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# Prunin Identification, Biological Activity and Quantitative Change in Comparison to Naringenin in Dormant Peach Buds[1,2]

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*Abstract.* Prunin (naringenin 7-glucoside), which was identified in dormant peach buds, was found to act as a growth inhibitor of wheat coleoptile elongation.

Quantitative estimation on a fresh weight basis of the flavonoid in peach buds revealed a high level in late summer, a steep decrease in autumn that became more moderate during the winter, with lowest values near bud swell in spring. Two periods of reaccumulation of prunin during October and January fitted 2 periods of arrested growth of the resting bud. A negative correlation was demonstrated between prunin and naringenin levels during August through October. It is proposed that prunin might act as a precursor for naringenin that accumulates in resting buds in autumn. It is suggested that the system glucoside-aglucone-$\beta$ glucosidase might have regulatory properties in the dormant bud.

Since the work by Hendershott and Walker (10) identifying naringenin (5,7,4′-trihydroxy flavanone) in dormant peach buds, much attention was drawn to the presence of flavonoid inhibitors in dormant organs.

Many workers have determined the presence of naringenin in dormant buds (1, 3), its biological activity (15, 16), and its endogenous quantitative change with time (1, 11, 19). The presence of various flavonoid compounds is well known in the wood of many *Prunus* species (9). In a preliminary report in 1966 (21) we suggested the presence of prunin in dormant peach buds. A confirmation of this was published recently (2). In this paper, further studies on the identification, quantitative change and a suggested function of prunin during dormancy, is reported.

## Materials and Methods

Dormant peach buds collected from 1-year-old shoots served as the plant material. Buds were either lyophilized and kept desiccated until examined, or examined immediately after harvest. The buds were pounded in cold (4°) ether, extracted 4 times for 30 min each time, and the supernatant fractions combined. When desiccated material was used, water-saturated ether was employed. The ether which extracted quantitatively both naringenin and another flavonoid compound, was shaken with water (pH 3.0) 4 times and the aqueous solutions combined. The unknown flavonoid was transferred to the aqueous phase while naringenin remained in the ether. Quantitative estimation of the unknown compound was done either by measuring the absorption of the acidic aqueous solution at 283 nm ($\lambda$ max) using a DU Beckman spectrophotometer, or by reading the absorption of the solution at pH 10.0 in a Klett Summerson colorimeter using filter No. 42 (transmittance limits: 390–450 nm).

A comparison between series of readings with both methods showed a very good correlation, $r = +0.96$. The optical density at 283 nm was found to give values 4.80 times greater than Klett units with the colorimeter. Standard error of this factor between the 2 methods was found to be $\pm 0.14$.

For comparison purposes, prunin was obtained by using Harborne's method (8) based on partial acid hydrolysis of commercial naringenin (Nutritional Biochemicals Corporation). The hydrolyzate was separated chromatographically on Whatman 3MM paper with 1-butanol:acetic acid:water, 40:11:29 (B:A:W). Prunin was identified at $R_F$ 0.73 by its fluorescence under UV light and ammonia vapors. Prunin appeared at the beginning of the hydrolysis and gradually diminished in its course, while naringenin accumulated.

The prunin spot was eluted from the paper with water and concentrated in a flash evaporator below 50° *in vacuo*. The concentrated solution was rechromatographed in NaCl 1 %, found at $R_F$ 0.43, eluted similarly and dried.

The molar concentration of prunin was determined by comparing naringenin released by complete acid hydrolysis of prunin with a standard curve of commercial naringenin obtained from Nutritional Biochemical Corporation.

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Acid hydrolysis was carried out according to Harborne (8). Aliquots taken after 10 and 30 min were separated on Whatman No. 1 paper with BAW 40:11:29. Sugars were located with an $AgNO_3$ spray and flavonoids by examination under UV light in the presence of ammonia vapors. Enzymic hydrolysis was carried out according to Hestrin *et al.* (12). β-Glucosidase (Mann Research Laboratory) was employed for an incubation period of 24 hr at 35°. Toluene was applied to prevent contamination by microorganisms. Aliquots of the tested solution were taken for chromatography before and after the end of the incubation period. Markers of glucose, rhamnose and galactose were used concomitantly. The paper was developed with 1-butanol:pyrimidine:benzene water (B:P:Be:W) 5:1:3:3, and after drying was treated with $AgNO_3$ to reveal reducing sugars.

Naringenin was extracted according to Hasegawa (9) with ether, evaporated to dryness and dissolved in hot benzene. An aliquot was chromatographed in B:A:W, 40:11:29. The naringenin spot was identified under UV light and ammonia, at $R_F$ 0.90, eluted with absolute methanol and the absorption determined at 288 nm (λ-max naringenin). Quantitative estimations were made according to a standard curve obtained with commercial naringenin.

The wheat coleoptile elongation test was carried out according to the method described by Hancock *et al.* (6) and modified by Gur and Samish (5).

## Results

Our indication of a new flavonoid compound in peach buds was obtained by a strong absorption in the UV range of an aqueous solution shaken with the original etheral extract of the buds. Examination of the absorption curve of this aqueous extract revealed λ-max at 281 nm a shoulder at 322 nm, and a minimum value at 248 nm. The curve was similar to known absorption curves of flavanones (14). Under UV and ammonia, yellowish fluorescence appeared, a light brown color was produced with $FeCl_3$, and $NaBH_4$ gave an orange-yellow color. These reactions led to the conclusion that the examined compound might be a flavanone. $R_F$ values of these compounds in comparison with naringenin, prunin and naringin were examined in 2 solvent systems. The values obtained, as well as the data reported in the literature, are shown in table I.

The unknown flavanone had an intermediate $R_F$ value between the aglycone naringenin and the diglycoside naringin in both solvent systems. These $R_F$ values correspond well with those of prunin, and it therefore seems that the unknown flavanone is a monoglycoside, possibly prunin, which was further tested by hydrolysis. Aliquots of the hydrolyzed and nonhydrolyzed tested material were chromatographed along with flavonoid markers and some sugars in B:A:W, 40:11:29 (table II).

Table I. *Experimental and Reported $R_F$ Values of the Unknown Compound, Prunin, Naringin, and Naringenin in 2 Solvent Systems*

| Compound | 1 % NaCl | | B:A:W 4:1:5 | |
|---|---|---|---|---|
| | Found | Reported in literature | Found | Reported in literature |
| | $R_F$ | $R_F$ | $R_F$ | $R_F$ |
| Unknown | 0.38 | ... | 0.69 | ... |
| Prunin | 0.43 | 0.44 | 0.68 | 0.64 |
| Naringin | 0.56 | 0.62 | 0.54 | 0.59 |
| Naringenin | 0.17 | 0.16 | 0.91 | 0.89 |

The unknown flavanone had the fluorescent color of prunin, as well as an almost identical $R_F$ value of 0.73. After hydrolysis for 30 min, which was still incomplete, a marked decrease in this spot was noted and a new spot at $R_F$ 0.90 appeared. This new value agrees with that of the aglycone naringenin. After hydrolysis, a spot of reducing sugar was found at $R_F$ 0.32, which was identical with that of glucose. Identical results were obtained by similar hydrolysis of a known standard of prunin. Enzymic hydrolysis of the unknown flavanone glycoside with β-glucosidase yielded glucose in the reaction solution, as identified after paper chromatography by treatment with $AgNO_3$ in alkali. One dark spot appeared having an $R_F$ value of 0.27 in B:P:Be:W, 5:1:3:3, identical with that of the marker glucose. Furthermore, color reactions identical with those of prunin were found under UV light and ammonia, and after reaction with $FeCl_3$, and $NaBH_4$.

In order to examine the position of the glucose on the aglycone, absorption curves of the isolated compound and of prunin in 95 % ethanol were determined. The changes in absorption after reaction with aluminum chloride or sodium acetate were followed (Fig. 1).

The bathochromic shift with $AlCl_3$ to a 291 nm peak points to a free hydroxyl in position 5. The ineffectiveness of sodium acetate on the absorption peak indicates that the hydroxyl in position 7 is

Table II. *$R_F$ Values of Hydrolysis Products of the Unknown Compound as Compared with Various Flavonoids and Sugars*

The chromatograms were developed with 1-butanol:acetic acid:water 40:11:29.

| Compound | Reference index values | |
|---|---|---|
| | Flavonoid | Sugar |
| | $R_F$ | $R_F$ |
| Unknown, not hydrolyzed | 0.73 | ... |
| " , hydrolyzed (30 min) | 0.73 ; 0.90 | 0.32 |
| Prunin | 0.74 | |
| Prunin, hydrolyzed (30 min) | 0.74 ; 0.90 | 0.32 |
| Naringin | 0.68 | |
| Naringenin | 0.90 | |
| Glucose | | 0.32 |
| Rhamnose | | 0.47 |

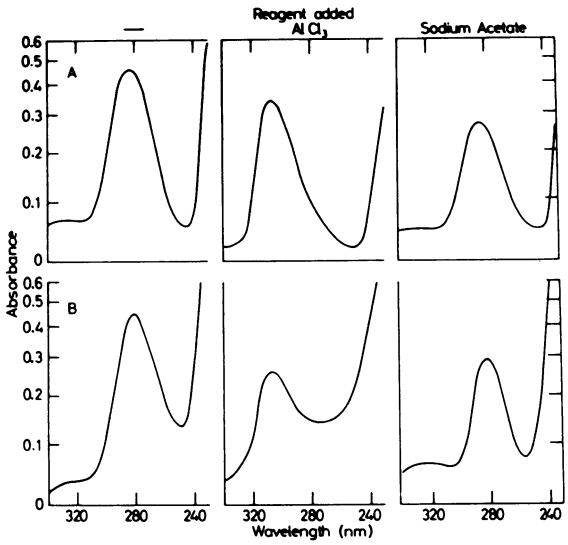


FIG. 1. Absorption curves in ethanol of prunin (A) and the isolated compound (B) and their change after reaction with aluminum chloride and sodium acetate.

glycosylated (13). Thus the identification of the flavanone as prunin was supported.

The biological activity of prunin was examined by using the wheat coleoptile straight growth test. The effect of various concentrations of a prunin extract from dormant Bonita peach flower buds sampled on September 11, 1964, after paper chromatography, is shown in Fig. 2. A pronounced inhibition, increasing with the concentration, was evident.

Examination of the seasonal change in prunin concentration was carried out by following the blue light absorption at pH 10 of the aqueous solutions

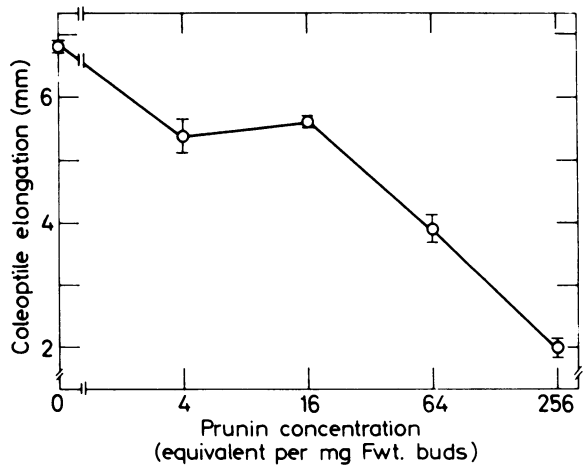


FIG. 2. The effect of varying concentrations of the prunin extract from dormant Bonita peach flower buds on the elongation of wheat coleoptiles. (Prunin spots were detected on the chromatogram under UV light and ammonia and the spot was cut out and used for bioassay. Sample for extraction was taken on September 11, 1964; initial coleoptile length was 12 mm.)

shaken with the original cold ether extract. A Klett Summerson colorimeter with filter No. 42 was used. The resulting data were plotted together with naringenin concentrations and fresh bud weight on corresponding dates (Fig. 3).

An overall decrease of prunin on a fresh weight basis was found from mid-August onwards. A slight reaccumulation, however, appeared during October and January. These periods of prunin

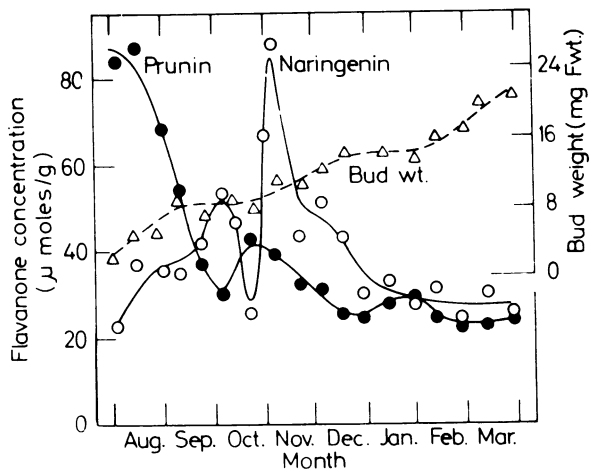


FIG. 3. The change in concentration of prunin and naringenin in Elberta flower buds during the dormant period in comparison with flower bud growth, 1961 to 1962 season. (Concn per fresh wt calculated according to a standard curve for naringenin and to naringenin liberated by hydrolysis of prunin for prunin; naringenin determination at 288 nm in methanol; prunin determination with a Klett Summerson colorimeter filter No. 42 in aqueous solution pH = 10.0.)

accumulation coincide with 2 quiescent stages found during the winter growth of the dormant peach flower bud. A negative correlation between prunin and naringenin concentrations was noted during August through October ($r = -0.723$ significant at 5% level). During this period, accumulation of naringenin was accompanied by a sharp decrease in prunin level.

Reexamination of the quantitative change of prunin concentration in dormant peach flower buds was done on samples collected weekly in 1962 to 1963, from December until bud break. Results were calculated on fresh-weight and single-bud bases and compared with bud growth (Fig. 4).

Our quantitative results for the season from December until bud opening, were confirmed during this season. Re-accumulation of prunin occurring during January preceded the halted growth of the flower buds. The decrease in prunin concentration in December and the increase in January were found also on a per-bud basis, thus indicating active changes of the compound in the buds. A decrease in prunin level on a fresh-weight basis was found during bud swelling while on pre-bud basis the level remained

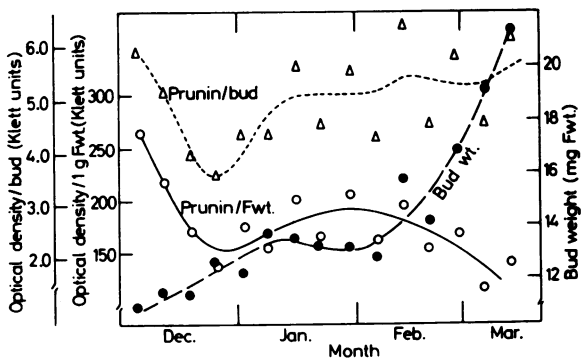


FIG. 4. The change in concentration of prunin in Elberta flower buds during the late dormant period, on the bases of fresh weight and single bud in comparison with flower bud growth in the 1962 to 1963 season. (Absorption of alkaline aqueous solutions, pH = 10.0, shaken originally with ether extract at pH 3.0; determined in a Klett Summerson colorimeter with filter No. 42.)

rather constant. Thus, the reduction in prunin concentration before bud break was mainly due to dilution.

## Discussion

The presence of prunin in dormant peach buds was reported by us (21) and by Corgan (2). This compound seems to be of biological importance, not only because of its inhibiting effect which is similar to that of the aglycone, but mainly in light of its high solubility in water. The high concentration of prunin in the bud found in August long before the rise in naringenin content, together with the negative correlation between the 2 compounds, mainly at the early half of the dormant season, seem to indicate that prunin might act as a precursor for naringenin. Prunin (as a $\beta$-glucoside) yields naringenin by hydrolysis and therefore a $\beta$-glucosidase should be involved in the reaction *in vivo*. Such enzymic activity was shown in dormant flower buds (4). It is therefore suggested that the system consisting of a sparingly soluble aglycone (naringenin), a highly soluble glucoside (prunin), and a hydrolytic enzyme ($\beta$-glucosidase), might regulate the growth inhibiting activity in the dormant bud. In such a system, the easily translocated prunin seems to play a more dominant role than naringenin, most of which is known to be accumulated in the bud scales (3).

It has already been suggested (17) that growth inhibitors inducing rest are synthesized in the leaves. Prunin, owing to its high water-solubility properties, is suggested to be the connecting link between the possible synthesis of the flavanone in the leaves and its deposition as an aglycone in the dormant bud and bud scales. The presence of prunin, together with $\beta$-glucosidase activity in the buds, may explain the rapid accumulation of naringenin in autumn, the local nature of the dormancy phenomenon (18), as well as the failure of exogenously applied naringenin to affect bud opening (3, 20).

It is suggested that prunin might act either alone or with naringenin as a causal inhibitor of growth since its rise preceded the halting of bud growth in most cases. However, the effect of exogenously applied prunin on bud growth has yet to be tested. Bud opening was not accompanied by a clear decrease in the inhibitor level, indicating the dependence on other regulating systems in this stage of development.

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