Identity and Occurrence of Certain Flavonol Glycosides in Four Varieties of Pears

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The principal flavonol glycosides of Packingham pears were isolated and identified. Mass spectrometry and nuclear magnetic resonance were used to confirm the identities of the aglycones. GLC and TLC were used to compare the aglycone and sugar moieties of hydrolyzed glycosides with known compounds. All compounds were characterized by UV spectrophotometry. Previously reported identities of quercetin 3-glucoside isolated from Bartlett and Bon Chretien pears and isorhamnetin 3-glucoside isolated from Bon Chretien pears were confirmed. Isorhamnetin 3-rutinoside, isorhamnetin 3-galactoside, and quercetin 7-xyloside were isolated from Packingham pears and identified. A compound of isorhamnetin substituted with another disaccharide of glucose and rhamnose at position three was also isolated. Two other glycosides of quercetin were observed; one contained substituents at the 3′ and 7 positions. Three other varieties of pears—Bartlett, Bosc, and D'Anjou—were compared.

Observed differences in chromatographic compounds of the phenolic patterns among pears, apples, and other fruit (Duggan, 1967; Nortjé and Koeppen, 1965; Sioud and Luh, 1966) are proposed for identifying fruit products. In earlier work (Duggan, 1967), the spots on chromatograms which gave positive color tests and UV spectra of flavonol glycosides appeared to be most promising for identification purposes.

Sioud and Luh (1966) observed the flavonol glucosides of Bartlett pears as two spots on paper chromatograms of extracts. They identified one of these spots as isoquercetin; the other they believed to be another glycoside of quercetin. Nortjé and Koeppen (1965) isolated five flavonoid compounds from Bon Chretien pears: isoquercetin, isorhamnetin 3-glucoside, a rhamnoglucoside of isorhamnetin, a rhamnogalactoside of isorhamnetin, and a fifth compound believed to be an acid-conjugated form of isorhamnetin 3-glucoside.

This paper reports the isolation and identification of flavonol glycosides from one variety of pears (Packingham), compares these glycosides with those of three other varieties, and relates these findings to previous work of other investigators.

EXPERIMENTAL

Procedures used in this work have been previously described (Duggan, 1967, 1969).

Materials. Packingham Triumph Pears were obtained from D. A. B. Grove of the Winchester Fruit Research Laboratory, Winchester, Va. Bartlett, Bosc, and D'Anjou pears were bought from commercial sources. The Packingham pears were held in −10° C. storage until analyzed. Samples of all varieties were also freeze-dried.

Extraction. Selection of a particular extraction procedure is based on the nature of the sample and the purity necessary for certain kinds of analyses. Extracts for the TLC comparison of flavonol glycosides in different varieties of pears were obtained by the methanolic extraction of homogenized fruit material followed by concentration, removal of interfering waxes by petroleum ether extraction, and partition of the flavonol glycosides into ethyl acetate. Further purification such as was used in the preparative TLC work was not attempted on these samples to preclude any selective loss of certain glycosides in precipitation or column procedures.

For preparative TLC, aqueous extracts were cleaned up by trapping the flavonol glycosides on a 15-cm. bed of polyamide (Woelm-Alupharm Chemicals) in a 3-cm. I.D. glass column. One column retains the flavonoids from about 500 grams of fruit. Free sugars were eluted with 200 ml. of water. Flavonoids were eluted with 75% ethanol. The alcoholic extract was concentrated, and the flavonol glycosides were separated by preparative TLC.

Extracts made primarily for the isolation of aglycones were purified by lead precipitation under controlled pH. Losses caused by solubility of lead compounds were minimized by keeping combined volumes of filtrate and washings within 40 ml. per 500 grams of fruit material.

Thin-Layer Chromatography. For TLC, glass plates (20 × 20 cm.) were coated with Avicel (microcrystalline cellulose, Brinkmann; or superfine, Food Machinery Corp.) and developed with 15:85 (v./v.) acetic acid in water (15HA) and/or phenol-water (4:1 w./w.). For two-dimensional TLC, the solvents were used in the order given and the plates were carefully dried between developments. The preparative TLC development with 15HA of plates streaked with extract resulted in separation of the material into two bands. These bands were scraped from the plates and the flavonoids were extracted with 70% aqueous methanol, concentrated, and streaked on fresh plates which were then developed in the phenol system. For comparing aglycones, the Forestal developing solvent (acetic acid–hydrochloric acid–water, 30:3:10, v./v./v.) (Seikel, 1964) was most frequently used.

Sugars were chromatographed against standards on 0.25-mm. plates of magnesium silicate (Woelm) activated at 120° C. for 30 minutes and developed for 5 to 6 hours with *n*-propanol–saturated aqueous boric acid–methyl ethyl ketone–acetone, 2:1:1:2 (v./v./v./v.) by the method developed by Zeeberg and Diaz (1969) in this laboratory, and were sprayed with anisidine or aniline phthalate.

Hydrolysis. One to 5 ml. of 0.1*N* HCl was added to fruit extracts. The solution was held at 100° C. until hydrolysis was complete (usually within 1 hour) as shown by the disappearance of the UV-absorbing flavonol glycoside spots and the appearance of yellow aglycone material on TLC plates. For removal of intact disaccharides from the glycosides, hydrogen peroxide was used as in the method of Chandler and Harper (1961).

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[1] Retired.

Gas Chromatography. A Barber-Colman Model 10 gas chromatograph was used with an argon ionization detector. Most of the work was done with an 8-ft. × 4-mm. I.D. glass column packed with 3% OV-17 on 100 to 120 mesh Chromosorb W-HP (Supelco, Inc.). Hydrolyzed samples were dried under vacuum at 40° C. and silylated by the addition of 2 ml. of pyridine, 0.5 ml. of hexamethyldisilazane, and 0.3 ml. of trimethyl chlorosilane. Excess solvent and reagents were removed by vacuum at 50° C. and the silyl derivatives were taken up in hexane for injection. Monosaccharides were chromatographed at a column temperature of 150° C., disaccharides at 235° C., and flavonol aglycones at 255° C. Retention times of sample peaks were compared with those of known compounds.

Spectrometry. For structure elucidation, ultraviolet spectra were taken in 95% ethanol with a Bausch and Lomb Spectronic 505 and a Cary 14 spectrophotometer. Sodium acetate, crystalline boric acid, aluminum chloride, and sodium ethylate were used as in the method of Jurd (1962).

NMR spectra of silylated samples in carbon tetrachloride were determined with Varian A-60 and HA-100 spectrometers and a C-1024 Time Averaging Computer.

Mass spectra of silylated aglycones were determined with an Atlas CH-4 spectrometer using a combination field-ionization electron-impact ion source.

RESULTS AND DISCUSSION

The flavonol glycosides of Packingham pear were characterized by two convergent lines of investigation: The number and identity of flavonol aglycones were determined by isolation from hydrolysates of pear extracts, and the flavonol glycosides, separated by a number of TLC procedures from fresh unhydrolyzed extracts, were individually characterized by UV spectrophotometry and TLC behavior in a number of solvent systems. Each isolated glycoside was then hydrolyzed and the aglycone identified by TLC and GLC comparison with the aglycones previously identified from whole extracts. The sugars were also identified by comparison with known compounds.

Aglycones. Hydrolysis of pear extracts yielded only two aglycones. The flavonoids were isolated as lead precipitates, regenerated with H_2S, hydrolyzed, and separated by preparative TLC on cellulose developed with Forestal solvent (Seikel, 1964). Their identities were established by MS, NMR, and UV spectrophotometric analyses. Mass spectra of their trimethyl silyl derivatives gave molecular weights of 604 and 662. The NMR spectrum of the silylated aglycone of molecular weight 604 matched very closely that of isorhamnetin (Mabry *et al.*, 1964). The other derivative was similar to that of quercetin; however, because of the small amount of sample available and the low signal-to-noise ratio, not all the peaks reported by Mabry *et al.* (1964) could be observed with certainty. The identification of isorhamnetin and quercetin corresponded to the findings of Nortjé and Koeppen (1965) for Bon Chretien pears.

After trial of many different GLC column packings, OV-17 on Chromosorb W-HP was found to give the best separation of the silylated aglycones. Figure 1 shows a gas chromatogram of the silylated hydrolysate of pear extract. The retention time of the major peak (C) corresponded to that for the purified material isolated from pears and identified as isorhamnetin by mass spectrometry and NMR; peak B corresponded to commercial quercetin.

Flavonol Glycosides. The number of separate compounds detectable in a natural product appears to depend upon the refinement of the separation procedure, the concentration of compounds, and the number actually present. The separation procedure was refined to the extent that nine different glycosides were obtained from Packingham pears and only one (P-7) gave evidence that it was still a mixture. A two-dimensional thin-layer chromatogram developed in the two systems formed the basis for final isolation procedures. P-8, which is not actually visible at the concentration spotted, is subject to the second limitation stated above. It appears on heavily streaked preparative plates. We do not feel that we have approached the third limitation. The compounds present in greater amounts have been identified: they are the ones encountered in the analytical procedures we propose for identifying fruit products (Duggan, 1969).

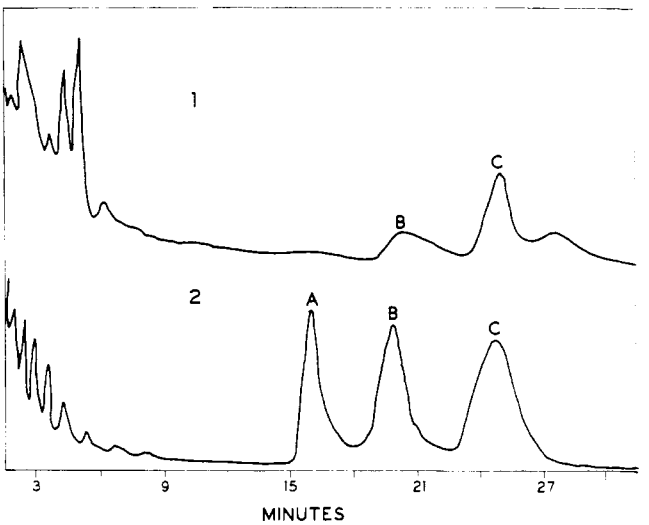

Figure 1. Gas chromatographic comparison of silylated hydrolysate of pear flavonol glycosides and of known compounds

Curve 1, pear; Curve 2, known compounds. (A) kaempferol, (B) quercetin, and (C) isorhamnetin

Using successive TLC separation of 1500 grams of material at a time, the glycosides were accumulated in amounts varying from 20 mg. to less than 1 mg. The tests performed were necessarily limited by the amount of material available. All the flavonol glycosides have been wholly or partially characterized by the standard UV tests for flavonoids (Jurd, 1962). Clues to the identity of the first six were also obtained by NMR.

Table I shows the shifts of principal absorption maxima upon the addition of certain reagents designed to show the class of compounds and position of substituents. Evidence for ortho dihydroxyls and the positions of substituents are incorporated into Table II, together with other data from NMR and from identification of hydrolysis products by TLC and GLC. Table II also compares the results of this work with those of previous investigators of other varieties of pears. The identities of isorhamnetin 3-glucoside and of quercetin 3-glucoside (isoquercetin) previously reported by other investigators were confirmed (P-1 and P-3). P-2, isorhamnetin 3-rutinoside, was isolated and the sugar identified, after its selective hydrolysis from the glycoside with hydrogen peroxide, by comparison with rutinose similarly hydrolyzed from rutin. This may be the compound previously reported by Nortjé and Koeppen (1965) as a compound of isorhamnetin substituted with rhamnose and glucose. P-4 contains a disaccharide of rhamnose and glucose attached at the 3-position of isorhamnetin. GLC and TLC data did

Table I. TLC and UV Absorption Properties of Pear Glycosides and Aglycones

Glycoside[a]	TLC R_f Value × 100 15HA	TLC R_f Value × 100 Phenol	Absorption Maxima, mμ		Shifts in Absorption Maxima (D Δmμ) upon Addition of Reagents: Sodium acetate		Sodium acetate and boric acid		Sodium ethylate		$AlCl_3$	
P-1	37	75	254	356	20	22	15	10	18	65	12.5	45
			256[b]	358[b]								
P-2	52	65	253	355	14[c]	8	12	8	16	62	12	47
P-3	34	45	255	358	18[c]	54	—[c]	42	14	56	14	52
P-4	53	76	256	357	14	7	14	6	17	63	13	46
P-5	37	82	254	355	16	23	11	9	17	66	10	45
P-6	45	40	255	357	1	6	5	27	1	1	17	65
P-7[d]	46	70	254	346	—	11	2	14	19	42	—	—
			253	348								
P-8	52	45	256[c]	360[c]	—	—	—	18	—	—		50
P-9	32	50	256	360	0	1	2	7	4	22	13	45
Aglycone												
P-1			253	368	21	24	0	2	20	−58	12	55
			255[b]	372[b]								
P-3			254	371	18	14	4	16	—	−40	12	56
			256[b]	374[b]								

[a] P-1 through P-9 refer to designations in text.
[b] Cary 14 Spectrophotometer; others in Bausch & Lomb Spectronic 505.
[c] Inflection.
[d] Unresolved mixture.

Table II. Summary of Experimental Results on Pear Flavonol Glycosides

Glycoside[a]	Free and Substituted Phenolic Hydroxyl Groups[b]: Ring A	Ring B	Ring C	CH_3—Group Present (by NMR)	Hydrolysis Products[c]: Aglycone TLC	Aglycone GLC	Sugar TLC	Sugar GLC	Conclusion
P-1	7 free	—	3 subst.	Yes	Isr	Isr	Glu	Glu	Isorhamnetin 3-glucoside[d]
P-2	7 free	—	3 subst.	Yes	Isr	Isr	Ru	Ru	Isorhamnetin 3-rutinoside[e]
P-3	7 free	free *o*-di(OH)	3 subst.	No	Q	Q	Glu	Glu	Quercetin 3-glucoside[d,f]
P-4	7 free	—	3 subst.	Yes	Isr	Isr	Disaccharide Rh Glu	Rh Glu	Isorhamnetin with disaccharide of rhamnose and glucose at 3 position[g]
P-5	7 free	—	3 subst.	Yes	Isr	Isr	Gal	Gal	Isorhamnetin 3-galactoside[g]
P-6	7 subst.	free *o*-di(OH)	3 free	No	Q	Q	Xyl	Xyl	Quercetin 7-xyloxide[g]
P-7	—	—	—	—	Q, Isr	Q, Isr	—	—	Mixture containing quercetin and isorhamnetin glycosides[e]
P-8	—	—	—	—	Q	—	—	—	Quercetin compound[g]
P-9	7 subst.	3 subst.	3 free	—	Q	—	—	—	Quercetin compound substituted at 7 and 3′ positions[g]

[a] P-1 through P-9 refer to designations in text.
[b] Based on UV absorption properties, Table I. Numbers refer to positions.
[c] Abbreviations: Isr, isorhamnetin; Q, quercetin; glu, glucose; ru, rutinose; rh, rhamnose; gal, galactose; xyl, xylose.
[d] Identified by Nortjé and Koeppen (1965) in Bon Chretien pears.
[e] May correspond to a compound previously found by Nortjé and Koeppen (1965) in Bon Chretien pears.
[f] Identified by Sioud and Luh (1966) in Bartlett pears.
[g] New compound not previously identified in pears.

not match those of rutinose, the only disaccharide of rhamnose and glucose available for comparison. P-5 was found to be isorhamnetin 3-galactoside; P-6, quercetin 7-xyloside. P-7 appeared to be an unresolved mixture, as its hydrolysate gave both isorhamnetin and quercetin upon GLC and TLC analysis. The TLC location of P-7 corresponds to that of a compound reported by Nortjé and Koeppen (1965). P-4, P-5, P-6, P-8, and P-9 are compounds not previously reported in pears. Identification of the aglycones of all of these compounds is given in Table II. P-8 and P-9 were recovered in such small amounts that no determination of sugars was possible.

Comparison of Other Pear Varieties. Two-dimensional TLC plates were prepared for three other varieties of pears: Bartlett, Bosc, and D'Anjou. Bartlett and Bosc differ from Packingham only in having slightly less P-4. D'Anjou differs most from the group. It contains much more P-6, much less P-5, and an extra compound which was isolated and found to be a compound of quercetin by TLC but was not further identified. Despite these differences, the TLC patterns of all of the pear extracts are characteristic of pears. The TLC and GLC methods used in this work and described in greater detail elsewhere (Duggan, 1969) have been shown to be useful for the identification of some fruits.

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