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PHENOLIC COMPOUNDS OF THE GENUS *PYRUS*: A CHEMOTAXONOMIC STUDY OF FURTHER OREGON SPECIMENS*

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Abstract—The leaf phenolics of a number of *Pyrus* specimens from the collection at Oregon State University, U.S.A., have been examined to extend a previous chemotaxonomic survey of *Pyrus*. Two unidentified phenolics, not previously found in *Pyrus*, have been found in certain of the specimens. *P. koehnei*, an E. Asian species not previously examined, has been found to be chemically very similar to *P. calleryana* in containing a number of phenolic acid esters of calleryanin (3,4-dihydroxybenzyl alcohol 4-glucoside). *P. ussuriensis* var. *hondoensis* has been found to lack luteolin and apigenin 4′-O-glucosides; this casts doubt upon its naming. Further specimens of *P. betulaefolia* have been found to lack flavone glucosides; it now seems that this E. Asian species lacks flavones and that earlier specimens were of hybrid origin.

INTRODUCTION

IN THE previous paper of this series[1] a chemotaxonomic survey was made of the leaf phenolics of *Pyrus* specimens from the Royal Botanic Gardens, Kew, and some specimens from the collection at Oregan State University, U.S.A. A second batch of *Pyrus* specimens (as grafting material) was obtained from Oregon in 1968, propagated at Long Ashton and the leaves were sampled later in the season. The present communication reports the results of this survey and a comparison is made with previous results.

RESULTS AND DISCUSSION

The occurrences of phenolic compounds, as found by two-dimensional paper chromatography of alcoholic leaf extracts, are listed in Tables 1, 2 and 3. The most important ways in which this second batch of Oregon *Pyrus* specimens (Oregon 2) differ from the previously examined specimens will now be discussed.

Previously, isochlorogenic acid was found to be completely absent from *P. salicifolia* var. *pendula* and most of the *P. calleryana* specimens; now it has been found in a different specimen of *P. salicifolia* var. *pendula* but not in *P. salicifolia* var. *orientalis*. It is also absent from two out of three specimens of *P. betulaefolia* and from one out of three specimens of *P. koehnei*. An unidentified cinnamic acid derivative (CP3), not previously found in *Pyrus*, has been found in the Oregon 2 specimens of *P. salicifolia* var. *pendula*, *P. betulaefolia* and a *P. betulaefolia* hybrid. When present, CP3 co-occurred with caffeoyl-calleryanin (CP1)[2] and on the standard SBA-2 per cent HAc two-dimensional paper chromatograms the CP3 spot

* Part of a thesis entitled "Studies on the Nature, Distribution and Biosynthesis of Phenolic Compounds in *Pyrus* and *Malus*" by J. S. Challice (July 1969).

The present paper is Part III of the series "Phenolic Compounds of the Genus *Pyrus*".

[1] J. S. CHALLICE and A. H. WILLIAMS, *Phytochem.* 7, 1781 (1968).

[2] J. S. CHALLICE and A. H. WILLIAMS, *Phytochem.* 7, 119 (1968).

TABLE 1. DISTRIBUTION OF FLAVONOIDS IN OREGON *Pyrus* SPECIMENS (2ND BATCH)

Rehder section No.	*Pyrus* species	Oregon specimen code	Geographical origin (Rehder)	Flavone glycosides**							Flavonol glycosides			catechins	
				F3	F2	F1	F4A	F4B	FZ	FS	F5	F6	F7	U1	U2
1	*P. amygdaliformis*	WB3	W. Asia, S. Eu.								+	+			
		Greece									+	+			
		WB7									+	+			
		WB1									+	+			
2	*P. salicifolia* var. *pendula*	MA	S.E. Eu., W. Asia								+	+	t?		
	P. salicifolia var. *orientalis*	MA									+	+	t?		
5	*P. communis* var. *pyraster*	MA	Eu., W. Asia								+	+			
		AA									+	+			
	P. syriaca	2	Desert shrub in Israel and Lebanon*								t	+			
		1									t	+			
?	*P. caucasica*	JR mag. seed									+	+			+
7	*P. ussuriensis*	Korea, E. Coast Mts.	N.E. Asia	+	+	t?	+	+		+	+			+	
		Seed 300 GD		+	+	t?	+	+		+	+				
	P. ussuriensis var. *hondoensis*	W6		+	+	+			+	+	+	t		+	+
9	*P. pyrifolia*	Korea Wild 3	C. and W. China		+	+			+	+	t	t			
		Donovan		+	+	+			+		+	t			
		Korea Wild 2		+	+	t			+		t?				

TABLE 1—*continued*

12	*P. betulaefolia* × *P.* (?)	Hybrid AA										+	+			
		Glen Dale 552		+	+				+			+	+			
	P. betulaefolia	Ansiloni Nurseries, Italy	N. China									+	+			
13?	*P. gharbiana*		N. Africa*	t	+		+	+	t			+	+		+	+
?	*P. koehnei*	UCLA 2	Extreme S. China and Formosa* (evergreen)		+											
		UCLA 1			+	t			t?							
		Gibbs			+	+			t?							
?	*P. kansuensis*	W6		+	+		+	+		+	t					

This table provides data which is intended to supplement the data in Challice and Williams (Ref. 1, Table 1).

** Flavone glycosides FO and FY (see Key) were found in trace amounts only in *P. gharbiana*; FX was not found in any of the above species.

Key: + = present, t = present only in trace amount, t? = doubtful trace, blank space = absent, – = presence not tested for. * = Information supplied by Professor M. N. Westwood. F3 = apigenin 7-glucoside, F2 = luteolin 7-glucoside, Fl = luteolin 7-rhamnoglucoside, F4A = luteolin 4′-glucoside, F4B = apigenin 4′-glucoside, FO = apigenin 7,4′-diglucoside (??), FY = luteolin 7,4′-diglucoside (??), FX = apigenin 7-rhamnoglucoside, FZ = chrysoeriol 7-glucoside, FS = unidentified spot, F5 = quercetin 3-monoglycoside complex, F6 = quercetin 3-diglycoside complex, F7 = quercetin 3-triglycoside complex, U1 = epicatechin (?), U2 = catechin (?).

TABLE 2. DISTRIBUTION OF CINNAMIC ACID DERIVATIVES IN OREGON *Pyrus* SPECIMENS (2ND BATCH)

Rehder section No.	*Pyrus* species	Oregon specimen code	Geographical origin (Rehder)	Cinnamic acid derivatives**													
				C1	C2	CP1	C3	C4	C5	C6	C7	C8	C9	C11	C12	CO	CP3
1	*P. amygdaliformis*	WB3	W. Asia, S. Eu.	t	t		+	+	+	t			t			–	
		Greece		+	+		+	+	+	+	t	t	+	+	+	–	
		WB7		+	+		+	+	+	+			t	t	t	–	
		WB1		+	+		+	+	+	+			+	t	t	–	
2	*P. salicifolia* var. *pendula*	MA	S.E. Eu., W. Asia	+	t	+	+	t	+	+			t	t	t	–	t
	P. salicifolia var. *orientalis*	MA					t	t	+	t				t	t		
5	*P. communis* var. *pyraster*	MA	Eu., W. Asia	+	+		+	+	+	+			+	+	+	–	
		AA		+	+		+	+	+	+			t	+	+	–	
	P. syriaca	2	Desert shrub in Israel and Lebanon*	+	+		+	+	+	t			t	t	t	–	
		1		+	+		+	+	+	t			t	t	t	–	
?	*P. caucasica*	JR mag. seed		+	+		t		+	+			+	t	t		
7	*P. ussuriensis*	Korea, E. Coast Mts.	N.E. Asia	+	+		+	+	+	+			+			t	
		Seed 300 GD		+	+		t	t	+	+			t			t	
	P. ussuriensis var. *hondoensis*	W6		+	+		+	+	+	+			+	t	t	+	
9	*P. pyrifolia*	Korea Wild 3	C. and W. China	+	+		+	+	+	+			+	t	t	t	
		Donovan		+	+		+	+	+	+	t	t	+	t	t	t?	
		Korea Wild 2		+	+		+	+	+	+			+	t	t	t?	
12	*P. betulaefolia* × *P.* (?)	Hybrid AA		+	t	+	t	t	+	+				+	+		+
		Glen Dale 552					t	t	+	+				t	t		
	P. betulaefolia	Ansiloni Nurseries, Italy	N. China			+	+	+	+	+				+	+	t?	+
13?	*P. gharbiana*		N. Africa*	+	+		+	+	+	+			t	t	t	+	
?	*P. koehnei*	UCLA 2	Extreme S. China and Formosa* (evergreen)			+			+	+				+	+		
		UCLA 1		t		+			+	+	+			t	t		
		Gibbs		t		+			+	+				t	t		
?	*P. kansuensis*	W6		+	+		t	t	+	+				+	+		

This table provides data which is intended to supplement the data in Challice and Williams (Ref. 1, Table 2).

** C1A was only found in UCLA 2 specimen of *P. koehnei*, C1B was not present in any of the above examined.

Key: + = present, t = present only in trace amount, t? = doubtful trace, blank space = absent, – = presence not tested for. * = Information supplied by Professor M. N. Westwood. C1 and C2 = *trans*- and *cis*-isochlorogenic acid, CP1 = *trans*-caffeoylcalleryanin, C3 and C4 = *trans*- and *cis*-caffeoylarbutin, C5 and C6 = *trans*- and *cis*-chlorogenic acid, C7 and C8 = *trans*- and *cis*-*p*-coumaroylarbutin, C9 = *trans*-neochlorogenic acid, C11 and C12 = *trans*- and *cis*-*p*-coumaroylquinic acid, C1A and C1B = *trans*- and *cis*-caffeic acid, CO = unidentified cinnamic acid derivative, CP3 = unidentified cinnamic acid derivative.

TABLE 3. DISTRIBUTION OF SIMPLER PHENOLS IN OREGON *Pyrus* SPECIMENS (2ND BATCH)

Rehder section No.	*Pyrus* species	Oregon specimen code	Geographical origin (Rehder)	Simpler phenols**											
				A1	H	A2	A3	X1	X2	P1	P2	P4	P6	P8	P5
1	*P. amygdaliformis*	WB3	W. Asia, S. Eu.	+	+	+									
		Greece		+	+	t?		t	t						
		WB7		+	+	t	t?								
		WB1		+	+	+		t	t						
2	*P. salicifolia* var. *pendula*	MA	S.E. Eu., W. Asia	+	+	+		t	t						
	P. salicifolia var. *orientalis*	MA		+	+	+		t	t						
5	*P. communis* var. *pyraster*	MA	Eu., W. Asia	+	+	+		t	t						
		AA		+	+	+		t	t						
	P. syriaca	2	Desert shrub Israel and Lebanon*	+	+	+									
		1		+	+	+									
?	*P. caucasica*	JR mag. seed		+	+	+		+	+						
7	*P. ussuriensis*	Korea, E. Coast Mts.	N.E. Asia	+	+		+	+	+						
		Seeds 300 GD		+	+			−	−						
	P. ussuriensis var. *hondoensis*	W6		+	+	t?		+	+						
9	*P. pyrifolia*	Korea Wild 3	C. and W. China	+	+	+	t	+	+						
		Donovan		+	+	+	t	t	t						
		Korea Wild 2		+	+	+									
12	*P. betulaefolia* × *P.* (?)	Hybrid AA		+		+		+	+						
		Glen Dale 552		+	+	+		t	+						
	P. betulaefolia	Ansiloni Nurseries, Italy	N. China	+	+	+		+	+						
13?	*P. gharbiana*		N. Africa*	+	+	+		t	t						
?	*P. koehnei*	UCLA 2	Extreme S. China and Formosa (evergreen)*	+						+	+	+	+	+	t
		UCLA 1		+						+	+	+	+	+	
		Gibbs		+	t					+	+	+	+	+	
?	*P. kansuensis*	W6		+	+	t	t								t?

This table provides data which is intended to supplement the data in Challice and Williams (Ref. 1, Table 3).

** P3 was found in trace amounts in the UCLA 2 specimen of *P. koehnei* compounds.

P2A and P7 were not present in any of the specimens examined.

Key: + = present, t = present only in trace amount, t? = doubtful trace, blank space = absent, − = presence not tested for. * = Information supplied by Professor M. N. Westwood. A1 = arbutin, H = hydroquinone, A2 = acetylarbutin, A3 = *p*-allylphenol (?), X1 and X2 = unidentified phenolics, P1 = protocatechuoylcalleryanin, P2 = vanilloylcalleryanin, P4 = *p*-hydroxybenzoylcalleryanin, P6 = protocatechuic acid 3-glucoside, P8 = calleryanin (3,4 dihydroxy, benzyl alcohol 4-glucoside), P5 = *p*-hydroxybenzoic acid, P2A = vanillic acid, P3 = protocatechuic acid, P7 = 3,4 dihydroxybenzyl alcohol.

is positioned just to the left of, and level with, CP1. The colour reactions of CP_3 were found to be: u.v. not visible, brilliant blue fluorescence under u.v. after NH_3-fuming, blue colour with Gibbs reagent. The evidence would be consistent with CP_3 being *p*-coumaroylcalleryanin. *P. koehnei*, which has not previously been examined, was found to contain high concentrations of the various phenolic acid derivatives of calleryanin;[2] in this respect *P. koehnei* is very similar to *P. calleryana*. The specimen listed as *P. kansuensis* in the tables is obviously a *Pyrus* species and should not be confused with the *Malus* species of the same name which is listed by Rehder.[3] In the Kew and first Oregon specimens the flavonol 3-mono and diglycoside complexes (F5 and F6) were absent only in the case of three Oregon specimens of *P. calleryana*; now both F5 and F6 have also been found absent from three specimens of *P. koehnei* and F6 alone absent from the second Oregon specimens of *P. ussuriensis*, *P. pyrifolia* and *P. kansuensis*.

An unidentified flavone glycoside (FS), not previously found in *Pyrus*, has been found in some new Oregon specimens of *P. ussuriensis*, *P. pyrifolia* and *P. kansuensis*. FS has an R_f value identical with luteolin 4'-glucoside (F4A) in both SBA and 2 per cent HAc (normal run) solvents but runs just below F4A (i.e. only slightly above the origin) on 2 per cent HAc (over-run 47 hr). The colour reactions of FS are: u.v. (~254 nm and ~366 nm) brilliant blue, yellow-green after NH_3-fuming; Gibbs reagent gave a brownish-grey colour (as given by chlorogenic acids). It is suspected that FS might be a flavone glycoside with the sugar moiety acylated by caffeic acid.

The fact that flavone 4'-O-glucosides are absent from *P. ussuriensis* var. *hondoensis* (Kikuchi and Nakai) Rehd. is sufficient to cast doubt upon whether this specimen is really a variety of *P. ussuriensis*. Once again, flavone glycosides have been found completely absent from Oregon specimens of *P. betulaefolia* (except for one hybrid); since Professor Westwood (private communication) has expressed confidence that his Oregon *P. betulaefolia* specimens are authentic species it would appear that the Kew specimen of this name is probably a hybrid. If this is so, then *P. betulaefolia* must be accepted as an E. Asian species which lacks flavone glycosides. *P. gharbiana*, examined for the first time with the second Oregon batch of specimens, seemed chemically very similar to *P. longipes*; both species come from N. Africa.

Westwood[4] has recently found that within the genus *Pyrus* there are selective degrees of resistance to the woolly pear aphid, *Eriosoma pyricola*; correlations between resistance and the presence of phenolics are not clear-cut although caffeoylcalleryanin might have some significance in this respect. Westwood has found considerable variability in degree of resistance within species; this indicates the necessity of parallel studies of resistance and phenolics present, at the level of particular specimens, if valid conclusions are to be drawn.

After most of the investigations were completed it was found that Catlin and Olsson had been investigating the occurrence of phenolic compounds in *Pyrus*;[5,6] their conclusions, based largely upon the distribution of unidentified phenolics, do not appear to conflict with our results.

EXPERIMENTAL

For experimental details, see the previous papers in this series.[1,2]

Acknowledgement—Thanks are due to Professor M. N. Westwood (Oregon State University) for very kindly providing the *Pyrus* specimens together with relevant taxonomic information.

[3] A. REHDER, *Manual of Cultivated Trees and Shrubs*, 2nd edition, Macmillan, New York (1954).
[4] M. N. WESTWOOD and P. H. WESTIGARD, *J. Am. Soc. Hort. Sci.* **94**, 91 (1969).
[5] P. B. CATLIN and E. A. OLSSON, *Proc. Am. Soc. Hort. Sci.* **88**, 127 (1966).
[6] P. B. CATLIN and E. A. OLSSON, *Proc. Am. Soc. Hort. Sci.* **93**, 88 (1968).