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PHENOLIC COMPOUNDS OF THE GENUS *PYRUS*—II.

A CHEMOTAXONOMIC SURVEY*

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Abstract—A paper chromatographic survey has been made of the leaf phenolics of all principal species of *Pyrus* and of one species of *Docynia*. The survey involved sampling of most species at different times during the growing season and showed that there is a basic pattern of phenolic constituents common to most of these plants. This basic pattern consists of arbutin, chlorogenic, neochlorogenic and isochlorogenic acids, a flavonol 3-monoglycoside complex, a flavonol 3-diglycoside complex, caffeoylarbutin (hitherto unreported in *Pyrus*), and a trace of *p*-coumaroylquinic acid. The flavonol 3-monoglycoside complex and caffeoylarbutin were found in higher concentrations in mature than in young leaf. Superimposed upon the basic pattern were found other phenolics showing selective distribution within the genus. These were luteolin and apigenin 7-glucosides and 7-rhamnosylglucosides (the latter two hitherto unreported in *Pyrus*); luteolin and apigenin 4′-glucosides (the latter hitherto unreported in *Pyrus*), chrysoeriol 7-glucoside (hitherto only suspected as occurring in *Pyrus*), two unidentified flavone glucosides both new to *Pyrus* (one of which may be luteolin 7,4′-diglucoside), a quercetin triglycoside complex, acetylarbutin, *p*-coumaroylarbutin (hitherto unreported in nature), a suspected 4-allylphenol (hitherto unreported in *Pyrus*), catechin and epicatechin. Calleryanin, its phenolic acid esters and the 4-glucoside of protocatechuic acid were found to be restricted to *P. calleryana*, except for caffeoylcalleryanin which occurred quite widely. The corresponding free phenolic acids were similarly restricted to *P. calleryana*, with the exception of *p*-hydroxybenzoic acid which also occurred in *P. pyrifolia*. Caffeoylcalleryanin was found in considerably higher concentrations in mature than in young leaf; the converse was found for the other calleryanin esters and protocatechuic acid 3-glucoside. *p*-Coumaroylarbutin, in contrast with caffeoylarbutin, occurred in much higher concentrations in young than in mature leaf. Flavone glycosides were found to be restricted to species from Rehder's sections 7–15 and to *Docynia*. All the flavone-containing species are listed by Rehder as originating in E. Asia, with the single exception of *P. longipes* which is said to come from Algeria.

INTRODUCTION

IN REHDER'S handbook,[1] the genus *Pyrus*, alongside seventeen other genera including *Malus*, *Sorbus*, *Crataegus*, *Cotoneaster*, *Docynia* and *Cydonia*, is placed in the subfamily Pomoideae of the family Rosaceae. Rehder lists the genus *Pyrus* as consisting of about twenty species; in England all cultivated dessert, culinary and perry pear trees are varieties of the common pear—*P. communis* L. In N. America, however, the cultivated pear is derived from two distinct sources: the European *P. communis* and the Oriental *P. serotina* Rehd. (actually listed by Rehder as *P. pyrifolia* (Burm.) Nakai). Although once of considerable commercial importance these hybrid pears, such as the Kieffer, have now largely been superseded by the Bartlett pear, a variety of *P. communis*. *P. nivalis* Jacq., commonly known as the snow pear, is said to be grown in parts of Europe, particularly in France, for the making of perry.[2] In England, perry pears are invariably varieties of *P. communis*, these forms being selected for factors

* Principally from a thesis entitled "A Comparative Phytochemical Study of the Genus *Pyrus*", by J. S. Challice (Nov. 1966).

[1] A. REHDER, *Manual of Cultivated Trees and Shrubs*, 2nd edition, Macmillan, New York (1954).

[2] L. H. BAILEY (editor), *The Standard Cyclopedia of Horticulture*, Vol. 3. Macmillan, New York (1930).

TABLE 1. DISTRIBUTION OF FLAVONOIDS IN THE GENUS *Pyrus*

Rehd. sect. No.	*Pyrus* species	Origin (Rehd.)	Source	Flavone glycosides									Flavonol glycosides			Catechins	
				F-3	F-2	F-1	F-4A	F-4B	F-O	F-Y	F-X	F-Z	F-5	F-6	F-7	U-1	U-2
1	*P. amygdaliformis*	W. Asia, S. Eu.	K										+	+	t	t	
	P. amygdaliformis var. *persica*	W. Asia	K										+	+	t		
	P. amygdaliformis var. *cuneifolia*		K										+	+	+		
	P. amygdaliformis × *P. nivalis* (*P. michauxii*)		K										+	+	t	t	+
2	*P. salicifolia* var. *Pendula*	S.E. Eu., W. Asia	K										+	+	t	+	t
	P. glabra	Persia	K										+	+	t		+
3	*P. elaeagrifolia*	Asia Minor	K										+	+	–	–	–
	P. elaeagrifolia WB4		O										+	+		+	+
	P. elaeagrifolia Olez 2		O										+	+		t?	+
4	*P. nivalis*	S. Eu.	K										+	+	t		
	P. nivalis × *P. salicifolia* (*P. canescens*)		K										+	+	t	+	
5	*P. communis*	Eu., W. Asia	K										+	+		+	+
	P. communis var. *cordata*		K										+	+		+	
	P. communis var. *jaspoideae*		K										+	+		+	
	P. communis × *Sorbus aria* (*Sorbopyrus auricularis*)		K										+	+		+	+
	P. communis × *Cydonia oblonga* (*Pyronia veitchii*)		K										+	+		+	
6	*P. regelii*	Turkest.	O										+	+	t		
7	*P. ussuriensis*	N.E. Asia	K	t	+	+	+	t	+	t		+	+	+			
8	*P. bretschneideri*	N. China	K	+	+							+	+	+	+		
9	*P. pyrifolia*	C. & W. China	K	+	+								+	+	+	+	+
	P. serotina var. *chozouri*		K	+	+	+					t		+	+	+		

10	*P. serrulata*	C. China	K	+	+	+						+	+	+	t	+	
11	*P. phaeocarpa* W6	N. China	O	t	+	+	t					+	+	+	t		
	P. phaeocarpa var. *globosa*	N. China	O										+	+	t	+	+
12	*P. betulaefolia*	N. China	K	+	+	t						+	+	+	+		+
	P. betulaefolia 1		O										+	+	+	+	
	P. betulaefolia 2		O										+	+	+	+	
	P. betulaefolia 3		O										+	+	t	+	
13	*P. longipes*	Algeria	K	+	+		+	+	+	t			+	+	t		
14	*P. pashia*	Himal., W. China	K	+			+	+	+				+	+	t	+	
	P. pashia 2		O	+	+	+	+	+	+				+	t			
	P. pashia 283		O				+	+	+				+	+			+
	P. pashia (India)		O										+	+			+
15	*P. calleryana*	China	K	+	+	t						t?	+	t			
	P. calleryana 2		O	+	+	+						t					
	P. calleryana 3		O	+	+	+						+					
	P. calleryana 8		O	t	+	+						t					
	P. calleryana var. *faurei* 1	Korea	O										+	+			
	P. calleryana var. *faurei* 4		O										+	+			
	P. calleryana var. *dimorphophylla* W6	Japan	O	+	+	+						+	t	t			
—	*Docynia delavayi*	S.W. China, Himal., Annam	K	+	+	+	+	+	t	t	+	+	+	+			

Key: + = present, t = trace amount, blank = absent, − = presence not tested for. K = Obtained from The Royal Botanic Gardens, Kew, England. O = Obtained from Professor M. Westwood, Oregon State University, Corvallis, U.S.A. F-3 = apigenin 7-glucoside, F-2 = luteolin 7-glucoside, F-1 = luteolin. 7-Rhamnosylglucoside?, F-4A = luteolin 4′-glucoside, F-4B = apigenin 4′-glucoside, F-O and F-Y = unidentified flavone glucosides (see text), F-X = apigenin 7-rhamnosylglucoside, F-Z = chrysoeriol 7-glucoside, F-5 = complex of quercetin monoglycosides, F-6 = complex of quercetin 3-diglycosides, F-7 = complex 3- of quercetin 3-triglycosides, U-1 = epicatechin?, U-2 = catechin?. For colour reactions, see Table 8 and Ref. 8.

TABLE 2. DISTRIBUTION OF CINNAMIC ACID DERIVATIVES IN THE GENUS *Pyrus*

Rehd. sect. No.	*Pyrus* species	Source	Cinnamic acid derivatives														
			C-1	C-2	CP-1	C-3	C-4	C-5	C-5	C-7	C-8	C-9	C-11	C-12	C-1A	C-1B	C-O
1	*P. amygdaliformis*	K	+	+	t	t		+	t			t	t	t			–
	P. amygdaliformis var. *persica*	K	+	+		+		+	t			t	t	t			–
	P. amygdaliformis var. *cuneifolia*	K	+	+	t	t		+	t			t	t	t			–
	P. amygdaliformis × *P. nivalis* (*P. michauxii*)	K	+	+		+	t?	+	t			t	t	t			–
2	*P. salicifolia* var. *pendula*	K			t			+	t				t	t			
	P. glabra	K	+	+		+	t	+	t			t	t	t			–
3	*P. elaeagrifolia*	K	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–
	P. elaeagrifolia WB4	O	+	t		+	t	+	+			t	t	t			t
	P. elaeagrifolia Olez 2	O	+	+		+	t	+	+			t	t	t			+
4	*P. nivalis*	K	+	+	+	+	t	+	t			+	t	t			–
	P. nivalis × *P. salicifolia* (*P. canescens*)	K	+	+	+	+	t	+	t			+	t	t			–
5	*P. communis*	K	+	+	t	+		+	t			t	t	t			–
	P. communis var. *cordata*	K	+	+	t	+	t	+	t			t	t	t			–
	P. communis var. *jaspoideae*	K	+	+		+	t	+	+			t	t	t			–
	P. communis × *Sorbus aria* (*Sorbopyrus auricularis*)	K	+	+		t		+	+			+	t	t			–
	P. communis × *Cydonia oblonga* (*Pyronia veitchii*)	K	+	+		+		+	t	t?		t	t	t			–
6	*P. regelii*	O	+	+		+	+	+	t	t		t	t	t			t
7	*P. ussuriensis*	K	+	+		t		+	+			t	t	t			+
8	*P. bretschneideri*	K	+	+		t	t	+	+	t?		t	t	t			+
9	*P. pyrifolia*	K	+	+	+	t		+	+			t	t	t			+
	P. serotina var. *chozouri*	K	+	+		+	t	+	+	+	t	t	t	t			+

10	*P. serrulata*	K	+	+	t	+	t	+	+	+	t	+	t	t			+
11	*P. phaeocarpa* W6	O	+	+		+	+	+	t	+?			t	t			
	P. phaeocarpa var. *globosa*	O	+	+	+	t		+	+				+	+			+
12	*P. betulaefolia*	K	+	+	+	t	t	+	+	+	t	t	t	t			+
	P. betulaefolia 1	O	t	t				+	+								
	P. betulaefolia 2	O	+	t		+	+	+	t	+	+	+					
	P. betulaefolia 3	O	+	+		+	t	+	t			t					
13	*P. longipes*	K	+	+	t	+	t	+	+			t	t	t			+
14	*P. pashia*	K	+	+	t	+	t	+	+			t	t	t			+
	P. pashia 2	O	+	+	+	+		+	+			+	t	t			
	P. pashia 283	O	+	+	t	t		+	+			t	t	t			t
	P. pashia (India)	O	+	+		+		+	+	+		t	t	t			t
15	*P. calleryana*	K	t		+			+	+			t	t	t	+	+	
	P. calleryana 2	O			+			+	+				t				t?
	P. calleryana 3	O			+			+	+				t				t?
	P. calleryana 8	O			+			+	+				t				
	P. calleryana var. *faurei* 1	O						+	+	+	+		t	t			+
	P. calleryana var. *faurei* 4	O	+	t				+	+	+	+		+	+			+
	P. calleryana var. *dimorphophylla* W6	O				+		+	+								+
—	*Docynia delavayi*	K	+	+		+?	t?	+	+	t?		t	t	t			

Key: + = present, t = trace amount, blank = absent, — = presence not tested for. K = Obtained from The Royal Botanic Gardens, Kew, England. O = Obtained from Professor M. Westwood, Oregon State University, Corvallis, U.S.A. C-1 and C-2 = *trans*- and *cis*-isochlorogenic acid, CP-1 = *trans*-caffeoylcalleryanin, C-3 and C-4 = *trans*- and *cis*-caffeoylarbutin, C-5 and C-6 = *trans*- and *cis*-chlorogenic acid, C-7 and C-8 = *trans*- and *cis-p*-coumaroylarbutin, C-9 = *trans*-neochlorogenic acid, C-11 and C-12 = *trans*- and *cis-P*-coumaroylquinic acid?, C-1A and C-1B = *trans*- and *cis*-caffeic acid, C-O = unidentified cinnamic acid derivative. Colour reactions, see Table 8 and Ref. 8.

TABLE 3. DISTRIBUTION OF SIMPLER PHENOLS IN THE GENUS *Pyrus*

Rehd. sect. No.	*Pyrus* species	Source	Simpler phenols														
			A-1	H	A-2	A-3	X-1	X-2	P-1	P-2	P-4	P-6	P-8	P-5	P2A	P-3	P-7
1	*P. amygdaliformis*	K	+	+	+		t	t									
	P. amygdaliformis var. *persica*	K	+	+	+		t										
	P. amygadliformis var. *cuneifolia*	K	+	+	+		t	t									
	P. amygdaliformis × *P. nivalis* (*P. michauxii*)	K	+	+	+		t										
2	*P. salicifolia* var. *pendula*	K	+	+	+		t										
	P. glabra	K	+	+	+		t										
3	*P. elaeagrifolia*	K	+	–	–	–	–	–								–	–
	P. elaeagrifolia WB4	O	+	+	+	+	t	t									
	P. elaeagrifolia Olez 2	O	+	+	+	+	t	t									
4	*P. nivalis*	K	+	+	+		t										
	P. nivalis × *P. salicifolia* (*P. canescens*)	K	+	+	+		t										
5	*P.communis*	K	+	+	+		t	t									
	P.communis var. *cordata*	K	+	+	+		t										
	P.communis var. *jaspoideae*	K	+	+	+		t										
	P.communis × *Sorbus aria* (*Sorbopyrus auricularis*)	K	+	+	+		t	t									
	P. communis × *cydonia oblonga* (*Pyronia veitchii*)	K	+	+			t	t									
6	*P. regelii*	O	+	+			t	t									
7	*P. ussuriensis*	K	+	+			t										
8	*P. bretschneideri*	K	+	+	+		t	t									
9	*P. pyrifolia*	K	+	+	+	+	t							+			
	P. serotina var. *chozouri*	K	+	+	+		t	t									

10	*P. serrulata*	K	+	+	+		t													
11	*P. phaeocarpa* W6	O	+	+	+															
	P. phaeocarpa var. *globosa*	O	+	+	+?	+	t	t												
12	*P. betulaefolia*	K	+	+			t													
	P. betulaefolia 1	O	+	+	t?			t												
	P. betulaefolia 2	O	+	+	+		t?	t?												
	P. betulaefolia 3	O	+	+	+?		t	t												
13	*P. longipes*	K	+	+	+		t													
14	*P. pashia*	K	+	+	t?		t	t												
	P. pashia 2	O	+	+	t?		t	t												
	P. pashia 283	O	+	+	+		t	t												
	P. pashia (India)	O	+	+			t	t												
15	*P. calleryana*	K	+	+		+			+	+	+	+	+	+	+	+	+			
	P. calleryana 2	O		+					+	+	+	+	+							
	P. calleryana 3	O		+					+	+	+	+	+							
	P. calleryana 8	O		+					+	+	+	+	+							
	P. calleryana var. *faurei* 1	O	+		+	+		+												
	P. calleryana var. *faurei* 4	O	+	t	+	+		+												
	P. calleryana var. *dimorphophylla* W6	O	+					+												
—	*Docynia delavayi*	K	+	+		+	t								t?					

Key: + = present, t = trace amount, blank = absent, — = presence not tested for. K = Obtained from The Royal Botanic Gardens, Kew, England. O = Obtained from Professor M. Westwood, Oregon State University, Corvallis, U.S.A. A-1 = arbutin, H = hydroquinone, A-2 = acetylarbutin (pyroside), A-3 = 4-allylphenol?, X-1 and X-2 = unidentified phenolic compounds, P-1 = protocatechuoylcalleryanin, P-2 = vanilloylcalleryanin, P-4 = *p*-hydroxybenzoylcalleryanin, P-6 = protocatechuic acid 3-monoglucoside, P-8 = 3,4-dihydroxybenzyl alcohol 4-monoglucoside (calleryanin), P-5 = *p*-hydroxybenzoic acid, P-2A = vanillic acid, P-3 = protocatechuic acid, P-7 = 3,4-dihydroxybenzyl alcohol. For colour reactions, see Table 8 and Ref. 8.

TABLE 4. ULTRAVIOLET SPECTRA OF COMPOUNDS ISOLATED FROM THE LEAF OF *Pyrus* SPECIES

Code	Source	Identification	λ_{max} (in nm) EtOH	+NaOAc	+NaOAc–H_3BO_3	+NaOEt M/500 (t = 1 min)	+NaOEt M/500 (t = 30 min)	+$AlCl_3$ 0·12% w/v (t = 5 min)
F-1	*P. serotina* var. *chozouri*	Luteolin 7-rhamnosylglucoside (?)	257 268i 357	267 421	263 382	270 407	270 407	275 405
F-2	*P. serotina* var. *chozouri*	Luteolin 7-glucoside	258 269i 355	263 418	262 382	267 410	267 410	277 410
F-4A	*P. longipes*	Luteolin 4′-glucoside	270 340	275 326i 353	272 341	273 385*	273 385*	283 294i 352 385i
F-X	*P. serotina* var. *chozouri*	Apigenin 7-rhamnosylglucoside	270 341	270 341 403i	269 345	274 405	274 407	278 301 346 394
F-3	*P. bretschneideri*	Apigenin 7-glucoside	269 336	268 344 400	269 340	267 397	271 400	278 299 345 385
F-4B	*P. longipes*	Apigenin 4′-glucoside	271 327	279 362	272 327	279 379*	279 379*	284i 299 339 388
F-Y	*P. longipes*	Unidentified flavone glucosides	270 338	270 338	270 338	272 385*	272 385*	280 293i 344 382
F-O	*P. longipes*	Unidentified flavone glucosides	272 330	279 355	272 330i	279 377*	279 376*	283 340 389
F-Z	*P. bretschneideri*	Chrysoeriol 7-glucoside	253 267i 348	255 267i 353 415	254 267i 350	261 269i 420	262 269i 420	263i 276 296i 357 395
F-5	*P. serotina* var. *chozouri*	Quercetin 3-monoglucoside + 3-monogalactoside	259 267i 365	273 385	263 383	275 417	273 413	272 368i 407
F-6	*P. serotina* var. *chozouri*	Quercetin 3-diglycoside complex	259 267i 364	271 391	263 385	275 415	275 415	273 365i 407
F-7	*P. serotina* var. *chozouri*	Quercetin 3-triglycoside complex	259 267i 355	274 402	266 382	276 413	276 413	276 358 410
C-1†	*P. serotina* var. *chozouri*	Isochlorogenic acid	245 310i 331	245 301i 333 385i	258 301i 357	264 314i 385	264 314i 382	245i 301i 334
C-3†	*P. serotina* var. *chozouri*	Caffeoylarbutin	245i 298 331	245i 295 334 385i	259 299 357	265i 310 383	265i 310 383	245i 298 332
C-5†	*P. serotina* var. *chozouri*	Chlorogenic acid	245i 303i 331	245i 303i 334 385i	259 308i 357	266i 313 385	266 311 383	245i 303i 333
C-9†	*P. serotina* var. *chozouri*	Neochlorogenic acid (?)	245i 300i 329	245i 300i 332 385i	259 307i 355	265i 311 382	265i 311 382	245i 300i 334
C-7†	*P. betulaefolia*	*p*-Coumaroylarbutin	225 300i 316	224 300i 317	300i 317	240i 312i 376	240i 312i 373	225 300i 316
F-2 aglycone	*P. ussuriensis*	Luteolin	255 267i 352	269 394	260 376	267 405	273 410	276 294i 360 392
F-Z aglycone	*P. ussuriensis*	Chrysoeriol	252 267 350	271 376	250 268 352	265 417	265 417	260 277 352 392

i = inflection.
* Marked drop in intensity of band, relative to that of original unshifted band.
† Isolated as the *trans*-isomer, equilibrated to mixture of *trans*- and *cis*-isomers by exposure to daylight.

TABLE 5. ULTRAVIOLET SPECTRA OF AUTHENTIC PHENOLIC COMPOUNDS

Compound	λ_{max} (in nm)																																			
	EtOH				+NaOAc				+NaOAc–H_3BO_3				+NaOEt M/500 (t=1 min)				+NaOEt M/500 (t=30 min)				+$AlCl_3$ 0·12% w/v (t=5 min)														
Luteolin 7-monoglucoside (Hoerhammer)	255	265i		351	257	265i	355	408i	259		376		267		408		—				274			427												
Apigenin 7-monoglucoside (Fluka AG)		270		341		268	341	398	268		339		267		398		—				277	300	348	386											
Chrysoeriol 7-monoglucoside (Horowitz)	253	270		350	259	270i	360	418	253	269	350		263		418	262		420		263i	278	295i	360	391											
Acacetin 7-rhamnosylglucoside (Ollis)		269		326		269	324		269		326		289		373*		—				278	300	339	385											
Quercetin 3-monoglucoside+3-monogalactoside	259	267i		366		276	395		263		385		275		420	274		418		275		424													
Quercetin 3-rhamnosylglucoside	259	267i		366		270	397		264		388		275		418	275		417		276		440													
Luteolin (Hoerhammer)	256	270		358		273	407		266		382		271		412	273		413		265i	276	355	395												
Apigenin (Fluka AG)		270		343		278	302i	380	272		347		278	332	407	278	333	405			280	303	347	390											
Diosmetin (Arthur)	254	272	291i	350		279	328	360	254	272	350		238i	274		391*	238i	275	391*		266i	280	296i	362	394										
Chrysoeriol (Horowitz)	251	269		350		273	325	365	410i	270		354		266	275i	415	266	275i	413		263	279	361	391											
Acacetin (synthetic)		270		327		278	295i	357	270		327		278	294i	370*		—				279	302	341	383											
Quercetin (Koch-Light)	256	268i	299	375	258	276	339	391	261	294i	389		247i		336	403		327	403i		272	319		443											
Chlorogenic acid (Fluka AG)†	247	304i		333	247i	304i	334	382i	258	310i	357		265		313i	385	265	313i	382		248	307i		337											
p-Coumaric acid (Fluka AG)†	227	299i		314		287	314i			287	314i				314i	340		312	334		231		320												

i inflection.
* Marked drop in intensity of band, relative to that of original unshifted band.
† Equilibrium mixture of *trans*- and *cis*-isomers.

such as high tannin content in the fruit. Apart from Rehder's description of the genus *Pyrus*, other botanical descriptions have been given by Reimer,[3] Rubtsov,[4] Kikuchi[5] and by Lee.[6] A taxonomic revision of the genus *Pyrus* is in the course of preparation by Professor Westwood of Oregon State University, U.S.A.

It is the purpose of this communication to survey the range of phenolic compounds present in the leaf of all available species of *Pyrus* and to compare the distribution of the chemical characters with the classification of species as given by Rehder. Some of the plant material used in the investigation was from botanic gardens and the authenticity of the individual species was not always completely established so any taxonomic implications arising from the distribution of chemical characters must be treated with reserve. The paper chromatographic survey revealed a number of unknown phenolics which were isolated from a limited range of species and identified by the usual means.

RESULTS AND DISCUSSION

(*a*) *Identification of Phenolic Compounds*

Tables 1–3 list the identities and distributions of the phenolic compounds occurring in the species examined. Table 4 gives the u.v. spectral data obtained from various phenolics newly isolated from Kew specimens of *Pyrus serotina* var. *chozouri*, *P. longipes* Coss. & Dur., *P. serrulata* Rehd. and *P. betulaefolia* Bge., together with the data obtained for the flavone glucosides of *P. ussuriensis* Maxim. and *P. bretschneideri* Rehd.[7] Some of the phenolics listed have already been characterized as a result of their occurrence in *P. calleryana* Dcne.[8] and in *P. communis*.[9] Table 5 lists u.v. spectral data of various authentic phenolic compounds used for comparison and Tables 6, 7 and 8 respectively list the hydrolysis products, R_f values and colour reactions of the phenolics isolated from the various sources. The data obtained from each isolated phenolic will now be considered.

F-1. This appears to be a luteolin rhamnosylglucoside. However, the spectral evidence for the position of attachment of the sugar moiety proved contradictory; whilst the $AlCl_3$ and borate shifts clearly indicated a 7-*O*-glycoside, the anomalous NaOAc shift (10 nm) of the low wavelength band did not support this contention. Since, however, this latter test has not always given reliable results in our experience, it would appear probable that F-1, as isolated from *P. serotina* var. *chozouri*, is in fact luteolin 7-rhamnosylglucoside. The NaOAc-induced shift of the low wavelength band, as a test for the presence of a free 7-OH in a flavonoid, relies upon the fact that conditions are sufficiently basic only for ionization of this particular grouping. Hence, a small change in basicity due to acid or alkaline contamination would be expected to negate the test; this has been found to be the case in a number of instances. Granted that the accepted concept of pH loses its proper significance when transferred from aqueous to alcoholic conditions, it would seem that some more rigorous control of pH is really necessary here is the test is to be relied upon.

[3] F. C. REIMER, Blight resistance in pears and characteristics of pear species and stocks. Oregon Agricultural College, Experiment Station Bulletin 214 (1925).
[4] G. A. RUBTSOV, *Am. Naturalist* **78**, 358 (1944).
[5] AKIO KIKUCHI, Specification and Taxonomy of Chinese Pears. Kyoto Univ. Japan, Collected Records of Hort. Research No. 3 (1946).
[6] S. H. LEE, *Proc. Am. Soc. Hort. Sci.* **51**, 152 (1948).
[7] A. H. WILLIAMS, *Chem. Ind.* 1318 (1964).
[8] J. S. CHALLICE and A. H. WILLIAMS, *Phytochem.* **7**, 119 (1968).
[9] A. H. WILLIAMS, *Phenolics in Plants in Health and Disease*, p. 3, Pergamon Press, Oxford (1960).

F-2. This fraction, as isolated from six E. Asian species of *Pyrus*, was characterized as luteolin 7-monoglucoside. Apart from the anomalous NaOAc shift (*ca.* 4 nm in each case), the spectral data clearly supported substitution of the 7-hydroxyl of luteolin by sugar.

F-4*A*. This fraction, from *P. longipes* and *P. ussuriensis*, was identified as luteolin 4′-glucoside. The marked drop in intensity of the long wavelength band in the presence of

TABLE 6. HYDROLYSIS PRODUCTS* OF COMPOUNDS ISOLATED FROM THE LEAF OF *Pyrus* SPECIES

		Acid hydrolysis	
Code	Source	Aglycone(s)	Sugar**
F-1	*P. serotina* var. *chozouri*	Luteolin	Rhamnose + Glucose (*ca.* 1:1)
F-2	*P. serotina* var. *chozouri*	Luteolin	Glucose
F-2	*P. longipes*		
F-2	*P. bretschneideri*		
F-2***	*P. ussuriensis*		
F-2***	*P. serrulata*		
F-4A	*P. longipes*	Luteolin	Glucose
F-4A***	*P. ussuriensis*		
F-X	*P. serotina* var. *chozouri*	Apigenin	Rhamnose + Glucose (*ca.* 1:1)
F-3	*P. serotina* var. *chozouri*	Apigenin + Luteolin (*ca.* 4:1)	Glucose
F-3	*P. longpipes*		
F-3	*P. bretschneideri*	Apigenin	Glucose
F-4B	*P. longipes*	Apigenin	Glucose
F-Y	*P. longipes*	Luteolin (v. weak)	Glucose
F-O	*P. longipes*	Insufficient for identification	Glucose
F-Z***	*P. ussuriensis*	Chrysoeriol	Glucose
F-Z***	*P. serrultata*		
F-5	*P. serotina* var. *chozouri*	Quercetin	Glucose + Galactose (*ca.* 1:1)
F-6	*P. serotina* var. *chozouri*	Quercetin	Glucose + Xylose + Galactose + Rhamnose (*ca.* 1:1:0·5:0·2)
F-6	*P. longipes*	Quercetin + trace Kaempferol	Glucose + Galactose + Rhamnose (*ca.* 1:0·2:1)
F-7	*P. serotina* var. *chozouri*	Quercetin	Glucose + Xylose + Rhamnose (*ca.* 1:0·2:0·2)
C-3†	*P. serotina* var. *chozouri*	Caffeic acid + Hydroquinone	Glucose
C-7†	*P. serotina* var. *chozouri*	*p*-Coumaric acid + Hydroquinone	Glucose
C-7†	*P. betulaefolia*		

* All hydrolytic fragments were characterized by paper chromatography in a range of different solvent systems alongside authentic specimens.

** Sugar ratios only very approximate—determined by visual assessment of *p*-anisidine HCl spot intensities.

*** Spectra of aglycones and authentic flavones compared (see Table 4).

† These compounds were unattacked by β-glucosidase, but gave arbutin, together with caffeic acid or *p*-coumaric acid, on $Ba(OH)_2$ hydrolysis.

NaOEt, relative to the intensity of the original band in EtOH, confirmed that the 4′-hydroxyl was substituted.

F-X. This fraction, isolated from *P. serotina* var. *chozouri*, was identified as apigenin 7-rhamnosylglucoside. The spectral evidence, including the lack of shift of the low wavelength band in the presence of NaOAc, fully supported this identification.

F-3. This substance, as isolated from four E. Asian species, was identified as apigenin 7-monoglucoside. As isolated from *P. serotina* var. *chozouri* and *P. longipes* it remained

unresolved from about one-quarter the amount of an unknown luteolin glucoside; from *P. bretschneideri* and *P. calleryana*,[8] it was found to be pure.

F-4B. This, as isolated from *P. longipes*, was identified as apigenin 4′-glucoside.

F-Y and F-O. These flavonoids, isolated from *P. longipes*, were not obtained in amounts sufficient for full identification. However, in view of the similarity of the Gibb's reagent colours with those of F-4A and F-4B, F-Y and F-O might possibly be the 7,4′-diglucosides of luteolin and apigenin respectively.

F-Z. This flavone glucoside, as isolated from *P. serrulata* and *P. bretschneideri*, was identified as chrysoeriol 7-glucoside. The aglycone (luteolin 3′-methyl ether) was clearly

TABLE 7. R_f VALUES OF COMPOUNDS ISOLATED FROM THE LEAF OF *Pyrus* SPECIES

Code	Identification	R_f values				
		2% HAc	Overrun 2% HAc*	SBA	50% HAc	PW
F-1	Luteolin 7-rhamnosylglucoside (?)	0·07	17·0	0·38	0·64	0·51
F-2	Luteolin 7-glucoside	0·02	6·5	0·51	0·53	0·57
F-4A	Luteolin 4′-glucoside	0·03	10·5	0·73	0·58	0·61
F-X	Apigenin 7-rhamnosylglucoside	0·14	—	0·59	0·76	—
F-3	Apigenin 7-glucoside	0·05	17·0†	0·70	0·66	0·78
F-4B	Apigenin 4′-glucoside	0·06	21·5	0·75	0·68	0·80
F-Y	Unidentified flavone glucoside	0·07	25·0	0·33	0·63	0·50
F-O	Unidentified flavone glucoside	0·09	33·5	0·45	0·68	0·44
F-Z	Chrysoeriol 7-glucoside	0·02	7·6	0·52	0·64	0·63
F-5	Quercetin 3-monoglycoside complex	0·14	36·0	0·70	0·66	0·49
F-6	Quercetin 3-diglycoside complex	0·30	off sheet	0·61	0·74	0·36
F-7	Quercetin 3-triglycoside complex	0·47	off sheet	0·44	0·79	—
C-1	*trans*-Isochlorogenic acid	0·14	—	0·79	0·74	0·34
C-2	*cis*-Isochlorogenic acid	0·23	—	0·79	0·74	0·34
C-3	*trans*-Caffeoylarbutin	0·38	—	0·80	0·78	0·72
C-4	*cis*-Caffeoylarbutin	0·46	—	0·80	0·78	0·72
C-5	*trans*-Chlorogenic acid	0·53	—	0·64	0·78	0·30
C-6	*cis*-Chlorogenic acid	0·70	—	0·64	0·78	0·30
C-9	*trans*-Neochlorogenic acid (?)	0·57	—	0·52	0·77	0·21
C-10	*cis*-Neochlorogenic acid (?)	0·71	—	0·52	0·77	0·21
C-7	*trans-p*-Coumaroylarbutin	0·57	—	0·87	0·83	0·84
C-8	*cis-p*-Coumaroylarbutin	0·74	—	0·87	0·83	0·84

* Solvent overrun for 45 hr, sheet 39 cm long, end serrated, figures refer to distance of spot (cm) from origin.
† At lower concentrations of F-3, its mobility dropped towards that of F-4A.

differentiated from its isomer diosmetin (luteolin 4′-methyl ether) by the behaviour of the long wavelength band in the presence of NaOEt. F-Z could only be separated from F-2 by use of the the 50 per cent HAc solvent.

F-5. From *P. serotina* var. *chozouri*, F-5 was found to consist of an unresolved mixture of quercetin 3-monoglucoside with quercetin 3-monogalactoside.

F-6. From *P. serotina* var. *chozouri* and *P. longipes* F-6 consisted of an unresolved mixture of quercetin 3-diglycosides. However, xylose was found only in the complex from the former species whilst an unresolved trace amount of kaempferol glycoside was found only in the latter species.

F-7. As isolated from *P. serotina* var. *chozouri*, this appears to be an unresolved complex of quercetin 3-triglycosides.

C-1 *and C*2. By chromatographic and spectral correspondence with authentic specimens, these were identified as *trans*- and *cis*-isochlorogenic acids.

C-3 *and C*-4. From *P. serotina* var. *chozouri* these were identified as *trans*- and *cis*-caffeoylarbutin. However, they did not correspond chromatographically with a specimen of 2-*O*-caffeoylarbutin obtained from Dr. E. Haslam. The fact that C-3 is resistant to β-glucosidase, together with the blue colour given by reaction with Gibb's reagent, provides good indication that the caffeoyl moiety is esterified with one of the sugar hydroxyls (2, 3,

TABLE 8. PHENOLIC COMPOUNDS OF THE GENUS *Pyrus*: COLOUR REACTIONS OF SPOTS AS DETECTED ON TWO-DIMENSIONAL CHROMATOGRAMS

Spot code	Ultra-violet light			Colour reagents	
	~254 nm	~366 nm	$AlCl_3$ ~366 nm	Diazo	Gibbs
F-1	dV	DNH_3Y	YNH_3Y	l. brown NH_3 nil	Green
F-4A	dV	DNH_3Y	YNH_3Y	R. brown NH_3 nil	d. Blue
F-4B	dV	DNH_3 nil	YNH_3Y	R. brown NH_3 nil	Violet
F-O	dV	DNH_3 nil	YNH_3Y	—	Violet
F-Y	dV	DNH_3 dull O	YNH_3Y	—	Turquoise
F-X	dV	DNH_3 dull Y	YNH_3G	l. brown NH_3 nil	Green
F-Z	dV	DNH_3 canary Y	YNH_3Y	l. brown NH_3 nil	Green
F-6	dV	DNH_3Y	YNH_3Y	l. brown NH_3 nil	Violet-d. Blue
F-7	dV	DNH_3Y	YNH_3Y	l. brown NH_3 nil	Violet-d. Blue
U-1	not vis.	not vis.	not vis.	l. brown NH_3 nil	Violet
U-2	not vis.	not vis.	not vis.	l. brown NH_3 nil	Violet
C-3, C-4	BNH_3G	BNH_3 G	—	R. brown NH_3 nil	Blue
C-5, C-6	BNH_3G	BNH_3G	—	brown NH_3 nil	Brown
C-7, C-8	$dVNH_3B$	not vis. NH_3B	—	l. brown NH_3 maroon	Blue
C-9	BNH_3G	BNH_3G	—	brown NH_3 nil	Brown
C-11, C-12	$dVNH_3B$	not vis. NH_3B	—	faint brown	Pale green
C-O	BNH_3G	BNH_3G	—	brown NH_3 nil	Brown
H	dV high conc. only	not vis.	not vis.	d. brown	Brown
A-2	dV high conc. only	not vis.	not vis.	l. brown NH_3 violet	Blue
X-1	nil	not vis.	not vis.	brown NH_3 violet	wk. Blue
X-2	nil	not vis.	not vis.	brown NH_3 violet	wk. Blue

Key; d = dark, V = violet, l = light, B = blue, G = green, NH^3 = ammonia fuming, nil = no change in colour, Y = yellow, wk = weak, med. = medium, R = reddish, O = orange, D = visible as a dark spot against a white fluorescent background.

4 or 6) as is the case with Haslam's compound.[10] The difference must lie in the position of acylation in the glucose molecule. As with caffeoylcalleryanin,[8] small amounts of the coumarin aesculetin were found to accompany the liberated caffeic acid in both acid and alkaline hydrolyses. Since caffeic acid is known to undergo facile *in vitro* ortho-hydroxylation followed by ring closure to aesculetin e.g.,[11] the aesculetin found in the hydrolysates was considered to be an artifact. By use of the specific colour reagent of Cartwright and Roberts,[12] quinic acid was shown to be absent from both acid and alkaline hydrolysates. Tests for the

[10] E. HASLAM, M. O. NAUMANN and G. BRITTON, *J. Chem. Soc.*, Suppl. 1, 5649 (1964).
[11] J. KAGAN, *J. Am. Chem. Soc.* **88**, 2617 (1966).
[12] R. A. CARTWRIGHT and E. A. H. ROBERTS, *Chem. Ind.* 230 (1955).

presence of free sugars in the alkaline hydrolysate were also negative. During the chromatographic purification of C-3 it was observed that an artifact was repeatedly produced; this appearing on the chromatograms as a spot having a brilliant blue fluorescence under u.v. light which turned pink upon ammonia-fuming. The R_f was zero in 2 per cent HAc and in SBA the spot ran just below the main C-3 compound; in 50 per cent HAc the artifact had an R_f of about one-half that of C-3. It proved impossible to obtain sufficient of this material for spectral examination.

C-5 and *C*-6. By chromatographic and spectral correspondence with an authentic specimen, these were identified as *trans*- and *cis*-chlorogenic acid.

C-7 *and C*-8. From *P. serotina* var. *chozouri* and *P. betulaefolia* these were identified as *trans*- and *cis*-*p*-coumaroylarbutin. The evidence did not clearly indicate whether the *p*-coumaroyl moiety acylated the sugar or the hydroquinone hydroxyl group. However, it would be reasonable to assume that the sugar is acylated, as in the case of caffeoylarbutin.

C-9 *and C*-10. The spectral data were found to be practically identical with those for chlorogenic acid. Comparison of the R_f values with those obtained by Scarpati[13] for the positional isomers of chlorogenic acid suggested that C-9 and C-10 are the *trans*- and *cis*-isomers of neochlorogenic acid.

(*b*) *Occurrence of Phenolic Compounds Within the Genus-General*

The identities and distributions of the phenolic compounds of *Pyrus* are given in Tables 1–3. Alcoholic extracts of leaf of all principal species of *Pyrus* listed by Rehder were examined, together with a species of the allied genus *Docynia* in which arbutin has been reported.[14] The specimens obtained from Kew were sampled on four separate occasions spread over a period of 18 months; those from Oregon were sampled once only (see Experimental). Figures 1–6 illustrate a selection of the two-dimensional paper chromatograms of the alcoholic leaf extracts; from these the chromatographic positions of all phenolics listed in the tables may be ascertained. On the illustrated chromatograms, spot F-Z (chrysoeriol 7-glucoside) is not shown separately since it coincides in position with spot F-2 (luteolin 7-glucoside). Further chromatograms run with the solvent pair SBA-50 per cent HAc were successful, however, in moving F-Z just ahead of F-2. It should be emphasized that in many cases the listed occurrence of individual phenolics in a number of different species, depends only on correspondence of R_f values and colour reactions; this can only be an indication, not an absolute proof, of identity.

(*c*) *Occurrence of Phenolic Compounds Within the Genus—Basic Constituents*

The survey shows that there is a basic pattern of phenolic constituents common to most *Pyrus* species; this pattern is shared by the single species of *Docynia* which was examined. The basic pattern consists of isochlorogenic acid, a flavonol 3-monoglycoside complex, a flavonol 3-diglycoside complex, caffeoylarbutin, chlorogenic acid, neochlorogenic acid, arbutin, Hydroquinone (artifact?), and *p*-coumaroylquinic acid (trace only). Of the Kew specimens, isochlorogenic acid appeared to be completely absent only from *P. salicifolia* var *pendula* Pall. whilst in the Oregon specimens, it was absent from all three specimens of *P. calleryana*, from one of the two specimens of *P. calleryana* var. *faurei* (Schneid.) Rehd. and from the only specimen of *P. calleryana* var. *dimorphophylla* (Mak.) Koidz. Caffeoylarbutin was only occasionally absent while chlorogenic acid and arbutin were always present. The

[13] M. L. SCARPATI and P. ESPOSITO, *Tetrahedron Letters* **18**, 1147 (1963).
[14] A. H. WILLIAMS, *Nature* **202**, 824 (1964).

quercetin mono- and diglycoside complexes were apparently absent only from the three Oregon specimens of *P. calleryana*.

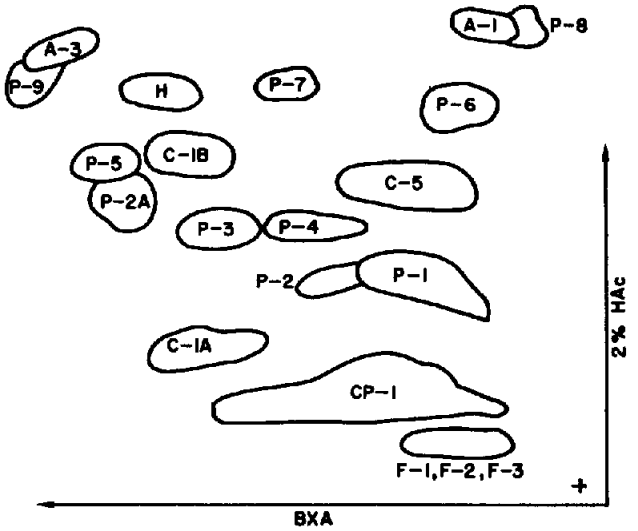

FIG. 1.

FIGS. 1–6. TWO-DIMENSIONAL CHROMATOGRAMS OF *Pyrus* LEAF EXTRACTS.

1. *P. calleryana*, OL-64
2. *P. nivalis*, OL-64
3. *P. pyrifolia*, OL-65
4. *P. serotina* var. *chozouri*, OL-64
5. *P. longipes*, OL-65
6. *P. longipes*, VYL-66

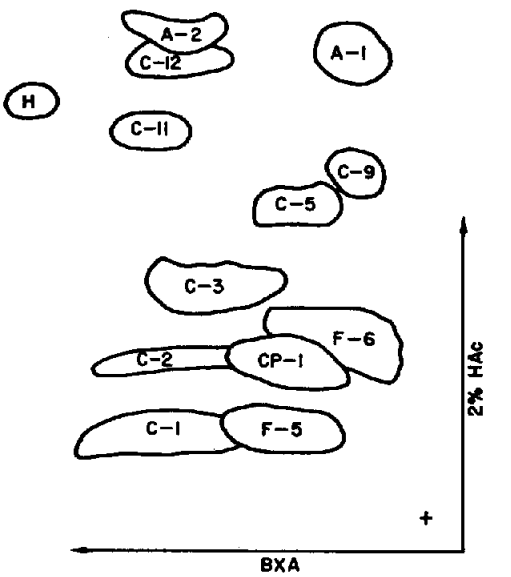

FIG. 2.

The results of the survey of Kew specimens indicated the desirability of sampling leaf material at different stages of growth throughout a season—otherwise some constituents,

could quite easily be missed. Of the basic phenolic constituents, the flavonol 3-monoglycoside complex (F-5) and caffeoylarbutin (C-3, C-4) were found in higher concentrations in the mature leaves than in the young leaves; with caffeoylarbutin the

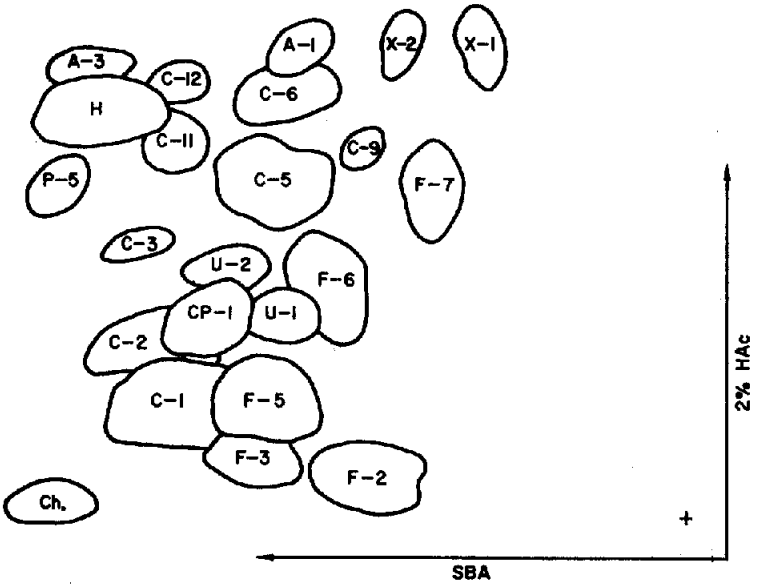

FIG. 3.

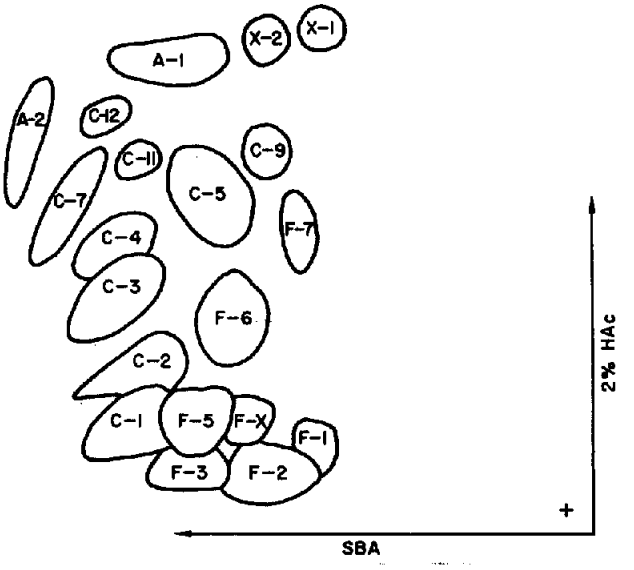

FIG. 4.

difference was found to be very pronounced. The remaining basic constituents did not display such obvious young–old leaf differences. Caffeoylarbutin has not previously been reported in *Pyrus*.

(d) Occurrence of Phenolic Compounds Within the Genus—Constituents With Selective Distribution

Superimposed upon the basic pattern of phenolic constituents are other compounds showing selective distribution within the genus. In no case does the basic pattern of phenolics

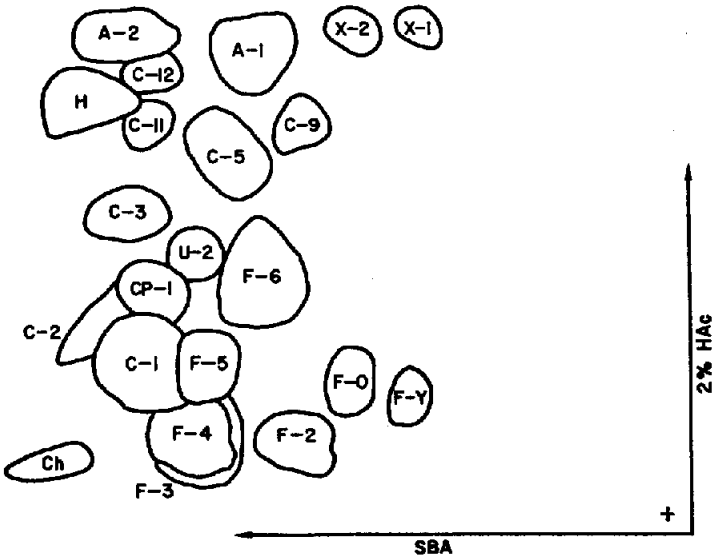

FIG. 5.

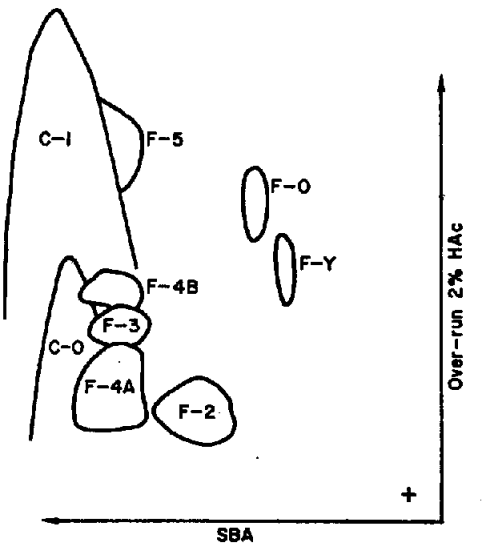

FIG. 6.

exist unaccompanied by these other constituents; every single species has a different combination of these rarer constituents. Naturally, not all of these constituents can be expected to provide reliably constant characters. Here again, some characters which appear to be

absent in a species may be there in amounts which are below the limits of detection; the sampling of leaf at different stages of growth should, however, reveal seasonal fluctuations and thus partly offset this. There is no guarantee that those constituents present below the limits of detection will not appear in detectable amounts in other specimens of the same species, especially if grown under different conditions; for example in the sunflower gentisic acid has been found to occur only under conditions of boron-deficiency.[15]

The flavone glycosides appear to provide the most fruitful source of chemotaxonomic characters in the genus *Pyrus*. Flavones were found only in the species in Rehder's sections 7–15; all these species come from E. Asia with the exception of *P. longipes* (section 13) which is said to originate in Algeria. However, since all known species of *Pyrus* are of the same chromosome number,[16, 17] inter-specific crossing is quite possible; hence the possibility of *P. longipes* being a hybrid between, say, *P. communis* and an E. Asian species cannot completely be ruled out. Certainly the leaves of *P. longipes* show a remarkable similarity to those of *P. communis*. In this connexion it is interesting that Rehder quotes an alternative authority for regarding *P. longipes* as a variety of *P. communis*—the occurrence of flavones certainly does not support this view.

It came as a surprise to find that, of the Oregon specimens of *Pyrus*, *P. phaeocarpa* var. *globosa* Rehd., all three specimens of *P. betulaefolia*, one of the three specimens of *P. pashia* D. Don. and the two specimens of *P. calleryona* var. *faurei* are completely lacking flavone glycosides. Both *P. betulaefolia* and *P. pashia* are generally regarded as primary species.

Westwood[18] considers that *P. calleryana* var. *faurei* is a distinct species, a native of Korea, and not a subspecies; the lack of flavone glycosides and calleryanin derivatives supports this view. Westwood also considers that *P. calleryana* var. *dimorphophylla* is a distinct form from Japan and not a subspecies; here flavone glycosides are present, but the complete absence of calleryanin derivatives appears to support Westwood's contention.

It is of interest that F-4A, F-4B, F-O and F-Y, when present, tend to co-occur; flavone 4′-*O*-glucosidation is present in the two former substances and is suspected in the latter two. Kikuchi[5] and Westwood[18] consider that both *P. phaeocarpa* and *P. bretschneideri* are hybrids of *P. betulaefolia* with either *P. ussuriensis* or *P. pyrifolia*; the presence of a trace of F-4A (luteolin 4′-glucoside) in one specimen of *P. phaeocarpa* would seem to indicate that this species is *P. betulaefolia* × *P. ussuriensis* and so *P. bretschneideri* is probably *P. betulaefolia* × *P. pyrifolia*. Reimer[3] and Kikuchi[5] say that *P. serrulata* is certainly of hybrid origin, most probably deriving from *P. pyrifolia* and *P. calleryana*; unfortunately the phenolic patterns of these three species do not appear to be of any assistance in proving or disproving this contention.

The two specimens *P. pyrifolia* and *P. serotina* var. *chozouri*, which are supposed to belong to the same species,[1] appear to differ so much both chemically and morphologically that one is led to suspect a case of mistaken identity here. The chemical differences as seen in the tables amount to as many as eight. The coloured illustration of *P. serotina* (syn. *P. pyrifolia*) as given by Hedrick[19] showed a far greater similarity to the specimen known at Long Ashton as *P. pyrifolia* than to that known as *P. serotina* var. *chozouri*; hence it would appear that the

[15] A. ZANE and S. H. WENDER, *J. Org. Chem.* **29**, 2078, 2812 (1964).
[16] S. ADATI, *Cytologia* **4**, 182 (1933).
[17] A. A. MOFFLETT, *Genetica* **15**, 511 (1934).
[18] M. N. WESTWOOD, private communication.
[19] U. P. HEDRICK, The Pears of New York, State of New York Dept. of Agriculture 29th Annual Report, Vol. 2, Part II. J. B. Lyon Co., Albany (1921).

latter specimen has been wrongly identified. There would appear to be a strong possibility of hybridization here.

The distribution pattern of chrysoeriol 7-glucoside, restricted of course to the E. Asian group, does not show any obvious significance. It is worthy of note that the position of methylation is the same as that of the isorhamnetin glycosides isolated from the fruit skin of a variety of *P. communis*[20] and from the young leaves of a variety of *Malus pumila* Mill,[21] and of vanillic acid, found in bound form in *P. calleryana*.[8]

Docynia delavayi (Franch.) Schneid, has the phenolic pattern with flavones typical of an E. Asian species of *Pyrus*. Taxonomically speaking, *Docynia* is considered to be more closely related to *Cydonia* which does not contain any flavone glycosides.

Reimer[3] has reported that the roots of both *P. calleryana* and *P. betulaefolia* show a particularly high degree of resistance to the Pear Woolly Aphis; this is interesting since the Kew specimens of these two species were found to contain particularly high concentrations of caffeoylcalleryanin in the leaf. The same two species have also exceptional resistance to leaf spot diseases.[22] However, further work would be necessary in order to establish whether or not a causal relationship exists.

Brunet[23] has reported the occurrence of 3,4-dihydroxybenzyl alcohol 4-*O*-β-D-glucoside as a tanning precursor in the cockroach; this is in addition to the already established presence of protocatechuic acid 4-*O*-β-D-glucoside.[24] The co-occurrence of these two glucosides, having the same glucosidation positions, contrasts with the situation in *P. calleryana* and *Prunus lusitanica* L. where there are differing positions of glucosidation of the two aglycones. Thus two different biosynthetic pathways may exist for the formation of calleryanin, one based upon the reduction of protocatechuic acid 4-glucoside (in the cockroach) and another, which probably parallels the schemes of Zenk[25] and Pridham[26] for the biosynthesis of salicin in *Salix*, in the *Pyrus* and *Prunus* species. It appears that arbutin may also occur in the beetle *Eleodes longicollis*,[27, 28] as the precursor of benzoquinone which is employed here as a defensive secretion. Other such co-occurrences of the same phenolic in the plant and insect worlds are possible.[27, 29]

The two catechins, U-1 and U-2, do not appear to show any systematic distribution; in all probability they are basic to the genus as bark constituents where they would reflect woodiness. Catechins are known to be present in much higher concentration in the bark of *Pyrus communis* varieties.[9] The one flavonol glycoside which showed a selective distribution—i.e. F-7 which appears to be a complex of quercetin 3-triglycosides—was found only in small amounts; in view of the close relationship between F-7 and the quercetin 3-mono- and diglycosides which are common to nearly all species, F-7 would not appear to be a reliable taxonomic character. Of the known cinnamic acid derivatives present, only CP-1 (caffeoylcalleryanin) and C-7 (*p*-coumaroylarbutin) displayed selective distributions. C-7 was found in markedly higher concentrations in the young than in the old leaf samples. This suggests

[20] B. K. Nortjé and B. H. Koeppen, *Biochem. J.* **97**, 209 (1965).
[21] A. H. Williams, *Ann. Rep. Long Ashton Res. Sta.* p. 36 (1964).
[22] R. L. Knight, Abstract Bibliography of Fruit Breeding and Genetics to 1960, *Malus* and *pyrus*. Commonwealth Agricultural Bureaux 1963.
[23] P. C. J. Brunet, *Endeavour* **26**, 68 (1967).
[24] P. W. Kent and P. C. J. Brunet, *Tetrahedron* **7**, 252 (1959).
[25] M. H. Zenk, *Phytochem.* **6**, 245 (1967).
[26] J. B. Pridham and M. Young, *Phytochem.* **6**, 462 (1967).
[27] J. Weatherston, *Quart. Rev.* **21**, 287 (1967).
[28] J. J. Hurst, J. Meinwald and T. Eisner, *Ann. Entomol. Soc. Am.* **57**, 44 (1964).
[29] L. M. Roth and T. Eisner, *Ann. Rev. Entomol.* **7**, 107 (1962).

that C-7 is the immediate biosynthetic precursor of C-3 (caffeoylarbutin) which conversely was found to occur in higher concentrations in the mature leaf samples. Thus *p*-coumaroylarbutin cannot be regarded as a reliable characteristic of any given species. It has not hitherto been reported as occurring in nature. CP-1 (caffeoylcalleryanin) was found in high concentrations in the Kew specimens of *P. nivalis*, *P. nivalis* × *P. salicifolia*, *P. Pyrifolia*, *P. betulaefolia*, in all specimens of true *P. calleryana* and in trace amounts in some other species. It was found to be present in markedly higher concentrations in mature than in the corresponding young leaf, but did not show any systematic distribution. Of the simpler phenolics listed in Table 3, the *p*-hydroxybenzoyl, protocatechuoyl and vanilloyl esters of calleryanin (P-4, P-1 and P-2 respectively), together with protocatechuic acid 3-glucoside (P-6), are entirely restricted to true *P. calleryana*. In contrast with CP-1, they appeared in somewhat higher concenticrations in young than in old leaf. With the sole exception of *p*-hydroxybenzoic acid (P-5) which was found also in *P. pyrifolia*, the free phenolic acids are also restricted to *P. calleryana*. It is probable that these free phenolic acids, together with calleryanin (P-8) and its aglycone (P-7), are in fact artifacts produced during leaf extraction. In a similar manner, hydroquinone is probably present only as an artifact of arbutin. Acetylarbutin (A-2) was found in most species but not in all samplings of any species in which it occurred. Generally the highest concentrations were found in the young leaf samplings; here it often appeared partially to replace arbutin itself. A-2 was not isolated for detailed examination, since Friedrich[30, 31] has already recorded its occurrence in a number of *Pyrus* species. The identity of A-2 in this study was indicated by chromatographic comparison with an authentic specimen of 6-*O*-acetylarbutin (pyroside) prepared by the method of Haslam *et al.*[10] It is of interest that whilst both Friedrich and Haslam *et al.* found 6-*O*-acetylarbutin in the leaves of *P. communis* and *Vaccinium vitis idaea* L., it has recently been claimed that it is the 2-*O*-acetyl isomer (isopyroside) that actually occurs *in vivo* in the leaves of *P. communis*.[32] A-3, which is possibly 4-allylphenol,[8] was found only occasionally and seemed to be more obviously present in young leaf; it may represent an artifact of lusitanicoside (4-allylphenol 1-rhamnosylglucoside) which would not be detected by the procedures employed in the present investigation; hence it is impossible to say, on the evidence available, whether or not A-3 is present in glycosidic form in other species. Two unidentified phenolics, X-1 and X-2, were found only in trace amounts; in view of the blue colours given with Gibb's reagent they appear to be monophenols.

EXPERIMENTAL

(*a*) *Leaf Sampling and Extraction*

The specimens from Kew were propagated at Long Ashton Research Station from budding wood obtained from Kew in 1961. In order that any seasonal variations in the phenolics might be observed, the leaf of each species was sampled on four separate occasions over a period of 18 months. These samplings were as follows: OL-64, mature leaf, 14–19 Oct. 1964; YL-65, young leaf, 16–17 June 1965; OL-65, mature leaf, 4 Oct. 1965; VYL-66, very young leaf (unopened growing points), 24 Mar. 1966. Herbarium vouchers were prepared from all the Kew specimens. The specimens from Oregon were very kindly supplied, as grafting material, by Professor M. N. Westwood, Department of Horticulture, Oregon State University, Corvallis, U.S.A., in March 1967, propagated at Long Ashton and the leaf sampled on 12 July 1967.

The leaf material (*ca.* 5 g) was covered with absolute alcohol within *ca.* 1 hr of the initial leaf sampling, boiled for 1 min and the whole left for at least 1 week. After homogenizing and filtering, the volume of the extract was adjusted so that 1 ml represented the extract of 0·5 g of fresh leaf.

[30] H. FRIEDRICH, *Pharmazie* **15**, 319 (1960).
[31] H. FRIEDRICH, *Naturwissenschaften* **48**, 304 (1961).
[32] G. ENTLICHER and J. KOCOUREK, *Arch. Biochem. Biophys.* **118**, 305 (1967).

(*b*) *Paper Chromatography of Extracts*

The leaf extracts, equivalent to 75 mg leaf, were separated on two-dimensional chromatograms (Whatman No. 1) using the following solvent pairs: A, (1st) *sec*-BuOH:HAc:H_2O, 70:2:28 (SBA), (2nd) 2% v/v HAc; B, (1st) SBA, (2nd) 2% v/v HAc over-run for 32 hr (end of sheet serrated); C, (1st) *n*-BuOH:Xylene:HAc:H_2O, 6:4:2:8 (BXA), (2nd) 2% v/v HAc; D, (1st) SBA, (2nd) 50% v/v HAc. Solvent pair A gave a good separation of the range of phenolics but was not of much value for separating the phenolics found particularly in *Pyrus calleryana*; solvent pair C was of more use here, though it did not separate the flavone glycosides from each other. Solvent pair B was necessary to separate F-4A, F-3 and F-4B. The flavone glycoside patterns were considerably clarified by use of this solvent pair; any spots running faster than C-1 disappeared off the end of the sheet. Solvent pair D was specifically designed in order to separate F-Z from F-2; none of the other systems resolved this pair of spots.

(*c*) *Identification of Unknown Phenolic Compounds*

Larger-scale alcoholic extracts were prepared from the mature leaves of Kew specimens of *P. serotina* var. *chozouri*, *P. longipes*, *P. serrulata* and from the very young leaves of the Kew specimen of *P. betulaefolia*. The extracts were fractionated on Whatman No. 17 and No. 3MM papers employing the usual range of solvents.[8]

(*d*) *Detection of Phenolic Compounds on Two-Dimensional Paper Chromatograms*

The resolved phenolics were visualized (Table 8) as follows: (i) Appearance under u.v.: both at 254 nm (Hanovia Chromatolite) and 366 nm (Hanovia Fluorescence II) both before and after ammonia-fuming; (ii) treatment with *p*-nitrobenzene diazonium fluoroborate reagent 7 and examination of colours both before and after ammonia-fuming; (iii) dipping in 1% w/v $AlCl_3$ in EtOH, and observation under u.v. (366 nm) before and after ammonia-fuming; and (iv) treatment with Gibb's reagent.[8] In addition to these tests, all OL-65 leaf extracts were run on one-dimensional chromatograms with SBA and 2% HAc and these were treated with $NaBH_4$–HCl reagent[33] in order to test for flavanones or dihydrochalcones; negative results were obtained. Two-dimensional chromatograms of *P. glabra* Boiss., *P. communis*, *P. pashia* and *P. calleryana* (all OL-65 samples) were run using the same system and all but *P. calleryana* were again negative; with *P. calleryana* a weak violet spot was observed in the region of the strong spot of protocatechuoylcalleryanin (P-1). Since dihydrochalcones are unknown in the genus *Pyrus*[14] it would appear that a flavanone might be present in trace amount in the leaves of this species. The detailed examination of this species made earlier,[8] however, did not reveal the presence of this component.

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[33] E. EIGEN, M. BLITZ and E. GUNSBERG, *Arch. Biochem. Biophys.* **68**, 501 (1957).