PHENOLIC ACIDS OF PLANTS AND THEIR ESTERS AND GLYCOSIDES

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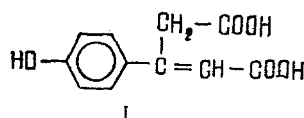
A review is given of the chemical structure of phenolic acids, their esters with alcohols, carbohydrates, anthocyans, and flavonoids, and their glycosides. Modern physicochemical methods of analysis are described. Color reactions of the phenolic acids on paper and thin-layer chromatograms, the solvent systems most frequently used for chromatographic analysis, and UV-spectral characteristics are presented in tables.

Phenolic acids are widely distributed in higher and lower plants. On the basis of the number and arrangement of the hydroxy groups in the benzene ring they can be divided into derivatives of mono-, di-, and trihydric phenols. However, some workers consider the majority of phenolic acids of plants as derivatives of benzoic and cinnamic acids [1, 2].

Derivatives of monohydric phenols include p-hydroxybenzoic acid, salicylic acid, p-hydroxyphenylacetic acid (Taraxacum officinalis), p-hydroxy-α-methylphenylacetic acid (Pterocarpus indicum), sphagnum acid (p-hydroxy-β-carboxymethylcinnamic) (Sphagnum magellanicum) (I), and a number of others [1-3].



The acids derived from dihydric phenols are more diverse in structure. In phenolic acids derived from pyrocatechol, the carboxy group is frequently located in the para position to a hydroxyl. These acids are important in the vital activity of plants and are the most widespread compounds in them. They include protocatechuic, vanillic, isovanillic, caffeic, piperonylic, veratric, ferulic, insoferulic, and other acids. These acids may be components of alkaloids, glycosides, and lignin. Some of them been found in plants in considerable amounts. Thus, in the skin of the garden onion, the protocatechuic acid content amounts to 2% [4].

In plants, caffeic and ferulic acids, which are most frequently regarded as derivatives of cinnamic acid, form esters with alcohols, amino alcohols, carbohydrates, and acids [2]. Combinations of caffeic acid with malic, tartaric, shikimic, lactic, and quinic acids are known. These include chlorogenic acid and its isomers, chicoric acid (from the species Cichorium, Lactuca), phaseolic acid (Phaseolus, Trifolium), rosmarinic acid (Lamiaceae species) and others [2]. Isoferulic acid is found in plants in the form of an ester with choline (Sibara virginica) [5].

Acids derived from hydroquinone and resorcinol have been least studied. They form component parts of lichen acids (orsellinic, everinic, olivetolcarboxylic, and rhizoninic acids) and gentisic acid.

Derivatives of pyrogallol include gallic acid, which is the structural material of tanning substances. In plants, as a rule, it is found in the form of glycosides. This group also includes meta-digallic acid, meta-trigallic acid, hexahydroxydiphenic acid and its lactone – ellagic acid – and syringic, eudesmic, and sinapic acids. The last-mentioned, just like isoferulic acid, is found in plants in the form of an ester with choline (sinapin).

Acids containing polyenic residues are known. For example, cortisamine from Corticum salicycum, containing seven conjugated double bonds, and piperic acid isolated from Piper species [1].

To isolate phenolic acids from plant extracts wide use is made of chromatography on columns of cellulose, silica gel, or polyamide, countercurrent distribution, and preparative chromatography on paper and in thin layers of adsorbent [7-13]. Methods have been described for separating and identifying phenolic acids with the aid of the gas-liquid chromatography of their trimethylsilyl or methyl derivatives [14, 15, 54]. Gas-liquid chromatography has been successfully combined with mass spectrometry [16].

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TABLE 1. Color Reactions of Some Phenolic Acids on Paper Chromatograms [18-20, 23, 38]

Acid	Color of the spots under the action of						
	UV light	UV light + NH_3	0.5 N KOH in CH_3OH	Gepfner's solution	Pauly's solution	$FeCl_3$	Millon's solution
p-Hydroxy-benzoic	Violet	—	—	—	Pale yellow	Yellow	—
Salicyclic	Blue	—	—	—	Yellow	Red-violet	Yellow
Protocatechuic	Violet	—	—	—	Red-gray	Black	—
Vanillic	White	—	—	—	Reddish	Yellow	—
Syringic	—	—	—	—	Pink	Brown	—
o-Coumaric	Reddish	—	—	—	Orange	Pale orange	—
p-Coumaric	Pale bluish violet	Dark violet	Greenish yellow	Pale yellow	Reddish	Yellow-orange	Red
Ferulic	Bluish violet	Bright bluish violet	Yellow brown	Yellow brown	Violet	Brown	Yellow
Isoferulic	Blue	—	—	—	Brick red	Red	Yellow
Sinapic	Bluish green	Bluish green	Pale yellow	Yellow brown	Bluish violet	Pink-yellow	—
Caffeic	Light blue	Light blue	Red-brown	Red-brown	Brown	Gray-green	Yellow
Chlorogenic	Blue	Green	Red	Red-brown	Yellow brown	Green	—
Neochloro-genic	Blue	Green	Red	Red-brown	Yellow brown	Green	—
Cryptochloro-genic	Blue	Green	Red	Red-brown	Yellow brown	Green	—
Gentisic	Light blue	—	—	—	Brown	Bluish violet	—

A number of color reactions exist which permit phenolic acids to be distinguished on paper and thin-layer chromatograms. The reagents listed below are used for this purpose.

1. Freshly-prepared diazo compounds in sodium carbonate solution: a) the Pauly reagent – diazotized sulfanilic acid [18, 23]; b) diazotized p-nitroaniline [19, 22]; c) diazotized benzidine [19, 22]; d) diazotized β-diethylaminoethyl p-aminophenyl sulfone (the Rose) reagent [22]; e) Diazol Rose O [21].

2. The Schweppe reagent: A solution of 2 g of glucose in 20 ml of water is mixed with solution of 2 ml of aniline in 20 ml of ethanol and the mixture is diluted with n-butanol to 100 ml. After being sprayed, the chromatograms are heated in a drying cabinet at 125°C for 5-10 min [23].

3. The Gepfner reagent: A 1% solution of sodium nitrite is mixed with an equal volume of 10% of acetic acid. After being sprayed and dried in the air, the chromatogram is treated with a 0.5 N methanolic solution of caustic soda [18, 20].

4. The Millon reagent: 100 g of mercury is dissolved in 100 g of nitric acid (d 1.40), and then 140 ml of water is added and the mixture is left for 24h. The clear solution is used for spraying. After treatment with the reagents, the chromatograms are first kept at room temperature for 5 min and are then heated to 95°C [23, 24].

TABLE 2. Color Reactions of Some Phenolic Acids on Thin-Layer Chromatograms [19, 23]

Acid	Adsorbent				
	silica gel			cellulose	
	color under the action of				
	UV light	Pauly's reagent	conc. H_2SO_4*	diazotized p-nitroaniline + Na_2CO_3	diazotized benzidine + Na_2CO_3
Salicylic	Blue	Yellow	Somewhat dark	—	—
p-Coumaric	Violet	Yellow	Brown-violet	Orange (blue)	Orange (blue)
Caffeic	Light blue	Brown	Gray-violet		
Ferulic	Bluish violet	Red-brown	Gray-violet	—	—
Isoferulic	Blue	Orange	Gray-violet	Pink (violet)	Orange (pink)
Protocatechuic	Violet			Orange (violet)	Yellow-orange (pink)
Vanillic	Light blue	Orange		Violet	
p-Hydroxybenzoic	Violet			Yellow (pink)	Yellow (pink)

*After spraying with sulfuric acid, the chromatograms are heated to 110°C.

5. A 1-2% methanolic solution of ferric chloride [18, 23] (Barton's reagent: $FeCl_3 + K_3Fe(CN)_6$, is rarely used).

6. The Folin—Denis reagent: a solution of tungstophosphoric acid. After being sprayed, the chromatograms are dried and are sprayed with a 0.5 N methanolic solution of caustic soda [18]. At the present time, this reagent is being replaced by the more sensitive Folin—Ciocalteu reagent [49].

7. The Folin—Ciocalteu reagent: 10 g of sodium tungstophosphate and 2.5 g of sodium molybdate are dissolved in 60 ml of water, and then 5 ml of 85% phosphoric acid and 10 ml of concentrated hydrochloric acid are added successively. The mixture is boiled under reflux for 10 h, then 15 g of lithium sulfate, 5 ml of water, and one drop of bromine are added and it is boiled again (for 15 min), and after cooling the solution is made up to a volume of 100 ml [49]. The chromatograms are first sprayed with a 20% aqueous solution of sodium carbonate and then, after slight drying, with the diluted reagent (before spraying, one part of the reagent is diluted with three parts of water).

8. A 0.5 N solution of caustic potash in methanol [18].

Thin-layer chromatograms are visualized with concentrated sulfuric acid [23].

Tables 1 and 2 give the color reactions of phenolic acids on paper and thin-layer chromatograms.

Aqueous acidic systems, aqueous alcoholic systems containing acid, systems containing hydrophobic solvents, and a number of others are used for chromatographic separation on paper. In the case of thin-layer chromatography, systems containing benzene and chloroform are used more frequently. E. Stahl recommends that silica gel should be impregnated with solutions of borax or sodium tungstate or molybdate [49]. A list of systems most frequently used is given in Table 3.

Phenolic acids can also be analyzed with the aid of electrophoresis [37].

In 1966, 6-O-galloylglucose and 3,6-digalloylglucose and representatives of esters of mono- and disaccharides with coumaric, ferulic, and caffeic acids were isolated for the first time from the rhizomes of Polygonum bistorta [25]. At the present time, esters of phenolic acids with carbohydrates are the most widespread of plant compounds. Thus, in 204 randomly selected plants the presence of 1-O-caffeoylglucose in 31% of the plants and of 1-O-p-coumaroylglucose in 5% of them has been established by chromatographic investigations [2].

The structures of the esters of phenolic acids are represented by formulas (II-IV).

TABLE 3. Solvent Systems Used for the Chromatographic Analysis of Phenolic Acids and Their Esters and Glycosides [18, 19, 23, 25, 30, 28, 31, 37, 51, 47, 53, 55]

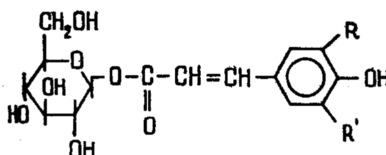
Solvent system	Ratio of the components of the system	For what compounds used
I. Paper Chromatography		
Conc. $HCl-H_2O$	3:97	Phenolic acids and their esters with sugars and anthocyans
$CH_3COOH-H_2O$	1:49; 15:85; 25:3	Phenolic acids, products of the alkaline cleavage of acylanthocyans
CH_3COOH— conc. $HCl-H_2O$	15:3:82	Acylanthocyans
Sodium formate—$HCOOH-H_2O$	10:1:200	Acylanthocyans
Butan-1-ol—85% $HCOOH-H_2O$	4:1:5	Phenolic acids
Butan-1-ol—2 N HCl	1:1	Acylanthocyans
Butan-1-ol—$CH_3COOH-H_2O$	4:1:5; 20:5:11; 4:1:2	Acylated O-glycosides of flavones and anthocyans, esters of phenolic acids with sugars, phenolic acids
Isobutanol—benzene—$HCOOH-H_2O$	100:19:10:25	Products of the alkaline hydrolysis of acylanthocyans
Butan-1-ol—pyridine—H_2O	6:4:3	Phenolic acids
Pentanol—$CH_3COOH-H_2O$	4:1:5	Esters of phenolic acids with sugars
Benzene—$CH_3COOH-H_2O$	2:2:1; 125:72:9; 6:7:3 (upper phase)	Phenolic acids
Toluene—$CH_3COOH-H_2O$	4:1:5; 5:1:4	"
Benzene—propan-2-ol—HCOOH	10:100:25	"
Xylene—butan-1-ol—acetone—H_2O	5:5:2:8; 8:2:2:5; 7:3:2:8	Phenolic acids
Xylene—butan-1-ol—$CH_3COOH-H_2O$	5:5:2:8; 8:2:2:8; 7:3:2:8	"
Chloroform—$CH_3COOH-H_2O$	3:1:1 (lower phase)	"
Ethyl acetate—$CH_3COOH-H_2O$	10:2:3	"
Butyl acetate—dioxane—1% HCOOH	68:30:2	Esters of phenolic acids with sugars
Methyl isobutyl ketone—$HCOOH-H_2O$	14:3:2	Hydroxycinnamic acids
II. Thin-layer chromatography. Adsorbent silica gel		
Chloroform—CH_3OH	1:1; 4:1; 9:1	Phenolic, acyl-O-glycosides of flavones
Benzene—CH_3OH-CH_3COOH	45:8:3	Phenolic acids
Benzene—dioxane—CH_3COOH	90:25:4	"
Ethyl acetate—methyl ethyl ketone—$HCOOH-H_2O$	5:3:1:1	Glycosides of phenolic acids
Benzene—acetone	3:1	O-Acylated flavonoid glycosides
Adsorbent cellulose		
$CH_3COOH-H_2O$	2:98, 15:85	Acylated C-glycosides of flavones and phenolic acids
0.1 N solution of HCl	—	Phenolic acids
CH_3COOH— conc. $HCl-H_2O$	25:3:72	"
Butan-2-ol—$CH_3COOH-H_2O$	70:5:25	"
Water-saturated di-n-butyl ether	—	"
Benzene—dioxane—CH_3COOH	90:25:4	"

(continued)

TABLE 3 (continued)

Solvent system	Ratio of the components of the system	For what compounds used
2 N NH_3−n-butanol	1 : 1 (upper phase)	"
Butanol -CH_3COOH—H_2O	4 : 1 : 5	Acylated C-glycosides of flavones
	Adsorbent polyamide	
Chloroform−CH_3COOH, saturated with water	3 : 2	Esters of phenolic acids with sugars
Methanol−H_2O	9 : 1	Acylated C-glycosides of flavones
Ethylacetate−CH_3COOH−(glacial)	95 : 5	Phenolic acids
30% CH_3COOH	—	"

Esters of phenolic acids with D-glucose:



I) R = R' = H → 1-O-(coumaroyl)-β-D-glucopyranose
III) R = OH; R' = H → caffeoyl-β-D-glucopyranose
IV) R = OCH_3; R' = H → O-1-feruloyl-β-D-glucopyranose.

The isolation of these compounds from the flowers, fruit and leaves of various species of the families Leguminosae, Solanaceae, Labiatae, Scrophulariaceae, Cruciferae, and other, have been described [22, 27]. Their structures have been shown by independent synthesis [26].

In UV light, these compounds are either colorless or have a blue fluorescence. After treatment with ammonia on chromatograms a blue or green fluorescence appears [31]. The hydrolysis of the esters takes place readily in 1% sulfuric acid at 100°C or with the aid of emulsin at pH 5.0 [26, 28].

UV and PMR spectroscopies are widely used for identification [20, 32, 34, 35, 52]. In neutral solutions, the UV spectra of the phenolic acids are usually identical with spectra of their esters, but on the addition of sodium acetate a hypsochromic shift of band (I) is observed, and on the addition of ethanolate a bathochromic shift [32]. In an alkaline medium, the spectra of the phenolic acid differ sharply from the spectra of their esters [33]. The UV spectral characteristics of these compounds are given in Table 4.

The UV spectral properties of the cis and trans isomers of hydroxycinnamic acids, which possess different physiological activities in plants, have been studied [34, 35], and a method of their electrophoretic separation has been developed [37]. It has been shown that it is predominantly the trans isomers that are present in plants [35], but a decrease in the amount of the trans form of ferulic acid and an increase in the amount of the cis form during the growth of maize seeds have been found [35].

The IR spectra of the hydroxyaromatic acids exhibit the characteristic bands of an aromatic ring and of a carboxy group and bands due to the vibration of free and associated phenolic hydroxyls and the cinnamic fragment.

In plants, phenolic acids also tend to form esters with alcohols, phenol glycosides, flavonoids, anthocyans (at the carbohydrate moiety of the molecule), sterols, triterpene compounds, and alkaloids [2]. Esters of phenolic acids with alcohols are found comparative rarely in plants. Esters of hydroxybenzoic, salicylic, gallic, and hydroxycinnamic acids with methanol and ethanol have been described. They have been detected in a number of fruits and vegetables with the aid of gas−liquid chromatography [2]. Esters of phenolic acids with alcohols having chain lengths of from C_{18} to C_{24} (eicosanyl ferulate, hexacosanyl caffeate, etc.) have also been detected in various organs of higher plants. Esters of caffeic and ferulic acids with hexacosane-1,6-diol, $HOCH_2-(CH_2)_{24}-CH_2OH$, have been isolated from oats [2]. Esters of caffeic, ferulic, gallic, and p-coumaric acids with glycerol are known [78, 79].

In a number of papers, Japanese workers have described esters of ferulic acid with β-sitosterol, cycloartanol, 24-methylenecycloartanol, cholesterol, stigmasterol, etc. Esters of hydroxycinnamic acids with ses-

TABLE 4. UV Spectral Characteristics of Some Phenolic Acids and Their Esters [2, 26, 31, 32, 53, 68]

Acid or its ester	λ, nm	Acid or its ester	λ, nm
I. Hydroxybenzoic acids			
Salicylic	207, 236, 305	Protocatechuic	217 sh., 258, 295
m-Hydroxybenzoic	210, 234, 298	Gallic	215, 271
p-Hydroxybenzoic	207, 253	Phloroglucinolcarboxylic	220, 261, 297
o-Pyrocatechuic	213, 246, 318		
β-Resorcylic	210, 256, 295	Vanillic	207, 216, 258, 290
Gentisic	212, 230 sh., 330		
γ-Resorcylic	216, 249, 317	Syringic	219, 272
α-Resorcylic	215, 250, 310		
II. Hydroxycinnamic acids			
o-Coumaric	214, 273, 325	Ferulic	217, 233, 297 sh., 320
m-Coumaric	214, 232, 276, 312 sh.	Isoferulic	217, 240, 292, 322
p-Coumaric	210, 223, 293 sh, 302 sh., 310	Sinapic	225 sh., 235, 322
Caffeic	217, 240, 297 sh., 325		
III. Acylquinic acids			
Chlorogenic	240, 325	3-p-Coumaroylquinic	250, 312
Neochlorogenic	245, 328		
1-Caffeoylquinic	245, 327	3-Feruloylquinic	325
4-Caffeoylquinic	246, 328	3-Isoferuloylquinic	326
Cynarin	245, 325		
3,5-Dicaffeoylquinic	329	3-Sinapoylquinic	327
IV. Esters of phenolic acids of mono- and disaccharides			
1-Feruloylglucose	237, 329	p-Coumaroylrhamnose	229, 312
Diferuloylsophorose	240, 324	p-Coumaroylglucose	229, 312
Feruloyl-p-coumaroylsambubiose	237, 327	p-Coumaroylrutinose	— 313
		p-Coumaroylsophorose	224, 313
1-p-Hydroxybenzoylglucose	260	1-Caffeoylglucose	247, 332
V. Acylated anthocyans			
Mathiolanin	283, 328, 509	Rubrobrassicin	282, 333, 530
Molardein	285, 314, 507	Hyacinthin	284, 310, 527
Salvianin	285, 329, 507	Petanin	282, 310, 538
Raphanasin C	278, 310, 523	Tibouchinin	280, 305, 536
Delphanin	282, 310, 538		
VI. Acylated C- and O-glycosides of flavones and flavonols			
2"-O-p-Coumaroylvitexin	212, 222 sh., 271, 318	6"-Sinapoylspinosin	275, 333
		6-Feruloylspinosin	276, 332
2"-Caffeoylisoorientin	250, 272, 298 sh., 337	Kaempferol 3-galloylglucoside	267, 290 sh., 350
2"-trans-Feruloylorientin	272, 297 sh., 331	Kaempferol 3-(6-galloylgalactoside)	267, 290 sh., 352
2"-trans-Feruloylorientin 4'-D- glucoside	273, 293 sh.,	Kaempferol 3-[di(p-coumaroyl)-D-glucoside]	268, 300, 314, 350
Apigenin 4'-O-p-coumaroylglucoside	268, 333	Petunoside	267, 5, 329
Apigenin 7-O-[6"-(p-coumaroyl)glucoside] (terniflorin)	269, 319	Kaempferol 3-O-[3"-O-p-coumaroyl)-6"-O-feruloylglucopyranoside	270, 300 sh., 320
		Myricetin 3-galloylglucoside	269, 293, 363

Note. The caffeic and ferulic acid derivatives have an inflection at 290-300 nm.

quiterpene alcohols (D- and L-chimgins, ferutinin, D- and L-chimganins, etc.) have been found in the roots of Ferula species [2].

Acylated anthocyans are more widespread in plants. The first acylated anthocyan was detected in plants in 1915. At the present time, it has been established that acylated anthocyans include residues of p-coumaric, ferulic, isoferulic, sinapic, and gallic acids, forming ester bonds with the carbohydrate moieties of the anthocyans.

To isolate these compounds, the raw material is extracted with aqueous ethanol or methanol with the addition of hydrochloric acid (1 ml to 1 liter of solvent). After the elimination of ballast substances, the acylglycosides are adsorbed on ion-exchange resins or are passed through a column containing polyamide [47, 30, 31]. Preparative chromatography of the eluates on paper is used for final purification. Partial acid hydrolysis is used to establish the structure of the acylcarbohydrate moiety. The acylated carbohydrate split out is identified chromatographically in the presence of markers obtained synthetically [30] or it is oxidized with hydrogen peroxide [31]. The structures of the acylated anthocyans in petunia flowers were established in this way [31]. To determine the structures of the acylglycosides petanin, negretin, and peonin use was made of periodate oxidation followed by reduction of the cleavage products with sodium tetrahydroborate [30]. Recourse is also made to exhaustive methylation of the acylanthocyans followed by hydrolysis and the study of the hydrolysis products. In this way, it was established that petanin, negratin, malvidin, and neopanin were 3[(4''-p-coumaroyl)rhamnosyl-(1 → 6)-glucoside] 5-glucosides and differed only in the structure of the aglycone.

Methylation is performed in dimethylformamide with methyl iodide in the presence of lithium hydroxide [30]. The separation of the phenolic acids is effected by hydrolysis with a 2N solution of caustic soda in a current of nitrogen at room temperature. Selective cleavage with hydrogen peroxide is also used. This method permits the detachment of acylated sugar residue linked to the third carbon atom of the acylanthocyan [31]. It is interesting to note that acylanthocyans are not hydrolyzed by the anthocyanase isolated from the fungus Aspergillus niger [56].

The UV spectrum of each of these compounds has an additional maximum at 310-355 nm, which is due to the superposition of the spectrum of the acylating acid on the spectrum of the anthocyan [37, 53]. The presence of a maximum at 308-313 nm is caused by p-coumaric acid, at 322 nm by ferulic acid, at 324 nm by sinapic acid, and at 326-328 nm by caffeic acid. The ratio of the acylation maximum and the maximum in the visible region is also important for these anthocyans. The value of this ratio depends on the number of acid residues present in the acylanthocyan molecule. Differential spectroscopy is also used to determine the acylating acid [53].

Acylated anthocyans can readily be distinguished from unacylated ones through their IR spectra. Thus, anthocyans acylated with p-coumaric acid or simultaneously with p-coumaric and caffeic acids have absorption bands in their IR spectra at 1690, 1600, and 830 cm^{-1}, which is characteristic for esters of 4-hydroxycinnamic acids [57].

The use of PMR spectroscopy to identify malvidin 3-(6''-p-coumaroylglucoside) isolated from the grape and to establish the position of the ester grouping has been described. Mass spectroscopy has been used to identify acylated monoglycosides, but it gives unsatisfactory results in the investigation of anthocyans [53].

Acylanthocyans have been detected in a number of families of dicotyledonous and monocotyledonous plants. The aglycones of these compounds are most frequently cyanidin, delphidinidin, and pelargonidin, and more rarely peonidin, petunidin, and malvidin. The UV-spectral characteristics of the acylanthocyans are given in Table 4.

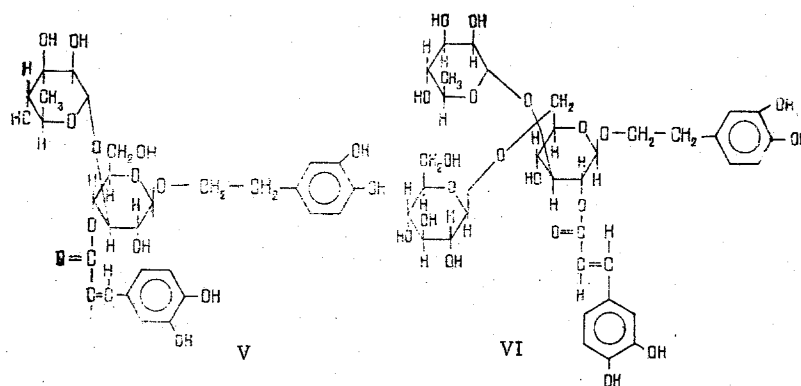
The first acylated flavonoid was isolated in 1961 from the flowers of the lime and was called tiliroside. It is kaempferol 3-O-(p-coumaroyl)-β-D-glucopyranoside. It has now been established with the aid of ^{13}C NMR spectroscopy that the p-coumaric acid residue is present in position 6'' of the glucose residue [59]. The structure has been confirmed by independent synthesis [80].

Flavone C-glycosides acylated with trans-ferulic, trans-caffeic, and p-coumaric acids and flavones and flavonol 1-glycosides containing gallic, p-coumaric, p-hydroxybenzoic, and ferulic acid residues are known. The aglycone of the acylated flavonoids are apigenin, luteolin, chrysoeriol, scutellarein, kaempferol, quercetin, isorhamnetin, syringetin, and myricetin [2, 48, 56]. To establish their structures, wide use is made of UV spectroscopy in the presence of ionizing and complex-forming additives and of PMR spectroscopy [48, 58, 60]. The cis-trans configuration of the cinnamic acid fragment is established with the aid of PMR spectroscopy [60].

The majority of acylflavonoids contain the phenolic acid residues in the carbohydrate components, but compounds are known in which the phenolic acid is linked directly to the aglycone. As examples we can give menthoside (4'-O-caffeoylapigenin 7-O-D-glucopyranoside), quinqueloside (4'-O-p-coumaroylapigenin 7-O-D-glucopyranoside), and 4-O-caffeoylscutellarein [61-64].

A less numerous group consists of glycosides of the phenolic acids. Thus, the β-glucosides of p-hydroxybenzoic, vanillic, and p-coumaric acids have been detected in the needles of Abies species [44]. The β-glycosides of vanillic and p-coumaric acids and the α-glucoside of p-hydroxybenzoic acid have been detected in the needles of the larch Larix faricina [42]. The 5-O-β-glucoside and a rhamnopyranosyl-(1 → 2)-β-glucopyranoside of gentisic acid have been found in Prunus yedoensis [2]. Trichocarpin and trichoside have been isolated from the bark of Populus species [3].

Glycosides of caffeic acid with a complex structure have been isolated from Syringa vulgaris [46]. One of them, acteoside – is β-(3,4-dihydroxyphenyl)ethyl O-α-rhamnopyranosyl-(1→ 3)-4-O-caffeoyl-β-D-glucopyranoside (V), and the other – neoacteoside – is β-(3,4-dihydroxyphenyl)ethyl [O-β-D-glucopyranosyl-(1→ 6)]-[O-α-L-rhamnopyranosyl-(1 → 3)]-2-O-caffeoyl-β-D-glucopyranoside (VI).



These glycosides may also exist in the form of cis and trans isomers, and under the influence of sunlight the trans isomer readily changes into the cis isomer [41].

At the present time, great attention is being devoted to the study of phenolic acids and their esters and glycosides. This is due not only to the fact that they are important in the vital activity of plants but also to the fact that a number of these compounds exert physiological and pharmacological action on the human and animal organism [2, 20, 71].

In view of this, a number of methods for the quantitative analysis of phenolic acids in plant material have been described [20, 72-77], and methods for the quantitative determination of phenolic acids in the presence of flavonoids have been developed [20, 74].

LITERATURE CITED

1. E. Soos, Pharm. Weekbl., 102, No. 15, 301 (1967).
2. K. Herrmann, Forschritte Chem. Org. Naturstoffe, 35, 73 (1978).
3. R. Tutschek, Pflanzenphysiol., 76, 353 (1975).
4. K. Hermann, Arch. Pharm., 291, 238 (1958).
5. R. Gmelin and A. Kjaer, Phytochemistry, 9, No. 3, 667 (1970).
6. O. E. Schultz and R. Gmelin, Z. Naturforsch., 76, 500 (1952).
7. J. R. Corse, R. E. Lundin, and A. C. Weiss, Phytochemistry, 4, 527 (1965).
8. M. L. Scarpati and G. Oriente, Tetrahedron, 4, 43 (1958).
9. R. Vancraenenbroeck and R. Lontie, Bull. Assoc. R. Anc. Etud. Brass., 70, No. 2, 71 (1984).
10. E. Kauppila, Suom. Kem., 31, No. 1, 82 (1958).
11. A. R. Guseva, Dokl. Akad. Nauk SSSR, 94, 1137 (1954).
12. C. Giovannozzi-Sermanni, Ric. Sci., 28, No. 9, 1871 (1958).
13. Z. Grodzinska-Zachwieja, W. Kahl, and A. Warchol, J. Chromatogr., 29, No. 2, 378 (1967).

14. M. Klimczak, Chem. Anal. (Warsaw), 22, No. 4, 645 (1977).
15. J. Wilson, W. J. Dunlap, and S. H. Wender, J. Chromatogr., 35, 329 (1968).
16. A. Blažej and L. Šuty, Phenolic Compounds of Plant Origin [Russian translation], Moscow (1977), p. 236.
17. H. Morita, J. Chromatogr., 71, 149 (1972).
18. K. Hermann, Pharmazie, 13, No. 5, 266 (1958).
19. F. LeScao, G. Faugeras, and R. Paris, Plant. Med. Pytother., 1, No. 3, 216 (1972).
20. N. V. Sergeeva, G. N. Zemtsova, V. A. Bandyukova, and A. L. Shinkarenko, Vopr. Pitan., No. 3, 54 (1973).
21. V. I. Kriventzow, Bull. Liaison – Groupe Polyphenols, No. 9, 308 (1980).
22. K. Kusnawidjaja, H. Thomas, and W. Dirscherl, Z. Ernährungwiss., 9, No. 4, 290 (1969).
23. H. Thiene and P. Benecke, Pharmazie, 25, No. 12, 780 (1970).
24. H. Thieme and R. Richter, Pharmazie, 21, 252 (1966).
25. F. Stinner and G. Korf, Arch. Pharm. 299, 640 (1966).
26. L. Birkofer, C. Kaiser, W. Nouvertné, and U. Thomas, Z. Naturforsch., 16b, No. 4, 249 (1961).
27. J. B. Harborne and J. J. Corner, Biochem. J., 81, 242 (1961).
28. W. Steck, Anal. Biochem., 20, No. 3, 553 (1967).
29. L. Birkofer, C. Kaiser, and H. Kosmol, Z. Naturforsch., 20b, No. 6, 605 (1965).
30. L. Birkofer, C. Kaiser, M. Donike, and W. Koch, Z. Naturforsch., 20b, No. 5, 424 (1965).
31. J. Harborne, Phytochemistry, 3, 151 (1964).
32. L. Jurd, Arch. Biochem. Biophys. 66, 284 (1957).
33. T. A. Geissmann and J. B. Harborne, Arch. Biochem. Biophys., 55, 447 (1955).
34. L. I. Dranik and T. A. Shubert, Dokl. Akad. Nauk SSSR, 185, No. 3, 705 (1969).
35. L. I. Dranik and T. A. Shubert, Khim. Prir. Soedin., 264 (1970).
36. T. A. Shubert, S. I. Pashkar', and F. I. Zemel', Fiziol. Rast., 16, No. 2, 308 (1969).
37. M. Metche, Contribution a l'Étude des Pigments Anthocyaniques chez Quelques Végétaux, Thès. Doct. Sci. Phys. Université de Nancy (1966).
38. H. Schmidtlein and K. Herrmann, Z. Lebensmitt.-Untersuch u.-Forsch., 159, No. 3, 139 (1975).
39. H. Thieme and R. Benecke, Pharmazie, 24, 292 (1969).
40. J. B. Harborne and V. H. Heywood, Biochem. J., 81, 242 (1961).
41. G. Kahnt, Naturwissenschaften, 49, 207 (1962).
42. H. J. Klosterman, F. Smith, and C. O. Clagett, J. Am. Chem. Soc., 77, 620 (1955).
43. H. J. Klosterman and R. Z. Muggli, J. Am. Chem. Soc., 81, 2188 (1959).
44. S. A. Medvedeva, S. Z. Ivanova, V. I. Lutskii, V. V. Keiko, and N. A. Tyukavkina, Khim. Prir. Soedin., 404 (1974).
45. G. J. Niemann, Phytochemistry, 8, No. 10, 2101 (1969).
46. L. Birkofer, Ch. Birkofer, and H. Thomas, Z. Naturforsch., 23b, No. 8, 1051 (1968).
47. L. Birkofer, Ch. Kaiser, W. Koch, M. Donike, and D. Wolf, Z. Naturforsch., 18b, No. 8, 631 (1965).
48. L. Birkofer, Ch. Kaiser, and F. Becker, Z. Naturforsch, 20b, No. 9, 923 (1965).
49. E. Stahl, Thin-Layer Chromatography, Allen and Unwin, London (1969).
50. P. Ribèrau-Gayon, Les Composés Phénoliques des Végétaux, Dunod, Paris (1966).
51. K. Herrmann, Pharmazie, 11, No. 7, 434 (1956).
52. L. Birkofer, Ch. Kaiser, B. Hilges, and F. Becker, Ann. Chem., 725, 196 (1969).
53. S. S. Tanchev, Anthocyans in Fruit and Vegetables [in Russian], Moscow (1980).
54. K. van de Casteele, H. Pooter, and C. F. Van Sumre, J. Chromatogr., 121, 49 (1976).
55. G. G. Zapesochnaya, S. Z. Ivanova, S. A. Medvedeva, and N. A. Tyukavkina, Khim. Prir. Soedin., 193 (1978).
56. J. Harborne, Comparative Biochemistry of Flavonoids, Academic Press, London (1967).
57. D. W. Anderson, D. E. Gueffory, A. D. Webb, and R. E. Kerner, Phytochemistry, 9, 1579 (1970).
58. L. Hörhammer, L. Stich, and H. Wagner, Arch. Pharm., 294, 685, 692 (1961).
59. V. M. Chari, H. Wagner, and A. Neszmelvi, Proceedings of the 5th Hungarian Bioflavonoid Symposium, Mitrafüred, Hungary (1977), p. 49.
60. Miah Duc Luong and A. Jacot-Guillarmod, Helv. Chim. Acta, 60, No. 6, 2099 (1977).
61. É. V. Gella, G. V. Makarova, and Yu. G. Borisyuk, Farm. Zh., 21, No. 3, 58 (1966).
62. É. V. Gella, G. V. Makarova, and Yu. G. Borisyuk, Farm. Zh., 22, No. 4, 80 (1967).
63. V. I. Petrenko, Khim. Prir. Soedin., 414 (1965).
64. V. I. Litvinenko and I. G. Zoz., Rast. Res., 5, 481 (1969).
65. R. Theodor, H. D. Zinsmeister, R. Mues, and K. R. Markham, Phytochemistry, 19, 1695 (1980).

66. L. Birkofer and Ch. Kaiser, Z. Naturforsch., 17b, No. 4, 279 (1962).
67. L. Hörhammer, L. Stitch, and H. Wagner, Naturwissenschaften, 46, 358 (1959).
68. L. I. Dranik, Khim. Prir. Soedin., 303 (1966).
69. H. Thieme and H. J. Winkler, Pharmazie, 23, No. 8, 1052 (1968).
70. H. Thieme and H. J. Winkler, Pharmazie, 26, No. 4, 235 (1971).
71. D. G. H. Daniels, H. G. C. King, and H. F. Martin, J. Sci. Food Agric., 14, 385 (1963).
72. C. Giovannozzi-Sermanni, Ricerca Sci., 28, No. 9, 1871 (1958).
73. K. Rauscher and J. Voigt, Ernährungforsch., 2, No. 4, 647 (1957).
74. M. Tissut, C. R. Acad. Sci., Paris, Série D, 270, No. 1, 205 (1970).
75. V. L. Kretovich and M. V. Uspenskaya, Biokhimiya Zerna, 2, 202 (1954).
76. A. R. Guseva and M. G. Borokhina, Biokhimiya, 20, No. 1, 106 (1955).
77. S. Pelvillani and V. Lattanzio, Conv. Intern. Polifenoli Assem. Annu., 1975, Groupe Polyphenols. Gargnano Garda (Milano), p. 1, 207 (1975).
78. Y. Asakawa and E. Wollenweber, Phytochemistry, 15, 811 (1976).
79. O. Schmidt and W. Blank, Chem. Ber., 89, 283 (1956).
80. B. Vermes et al., Helv. Chim. Acta, 64, No. 6 (1964).

CARBOHYDRATES OF Peganum harmala

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The carbohydrate complex of the epigeal part of Peganum harmala L. includes mono- and oligosaccharides, water-soluble polysaccharides, hemicelluloses, and an acidic polysaccharide, similar to the pectin substances of higher plants. It is based on a fragment constructed of α-(1→4)-linked D-galacturonic acid residues in the pyranose form.

In the present paper we give the results of an investigation of the carbohydrates isolated from the epigeal part of Peganum harmala L. (harmel peganum) collected in the flowering phase in May, 1981 in Dzhizak province, UzSSR. The air-dry raw material was treated with 96% ethanol to eliminate low-molecular-weight compounds and pigment substances. The mono- and oligosaccharides were isolated by extraction with 80% ethanol, and they were found by PC to include galactose, glucose, fructose, and sucrose. From the residue of the raw material, the water-soluble polysaccharides [1], pectin substances [2], and hemicelluloses [3] were isolated successively.

The amounts of the polysaccharides and their monosaccharide compositions according to PC and GLC [3] are given below (% on the air-dry mass of the plant):

Type of polysaccharide	Yield	*Gal*	*Glc*	*Man*	*Xyl*	*Ara*	*Rib*	*Rha*
Water-soluble	3,8	6,5	1,5	1	Tr.	7,8	Tr.	2,8
Pectin substances	4,8	1,3	1,0	—	Tr.	4,2	Tr.	1,4
Hemicelluloses								
A-1	5,0	1	6,3	Tr.	3,4	1,6	Tr.	1,4
B-1	4,28	9,9	36,5	2,2	9,0	10,0	1	10,0
A-2	2,56	3,3	7,6	2	9	5,6	Tr.	1
B-2	2,18	6	17,2	6,9	5,6	2,4	1	1,2

The water-soluble polysaccharide consisted of a white powder not giving a blue coloration with iodine, i.e., it did not contain a glucan of the starch type. D-Galactose and L-arabinose predominated in its hydrolysate.

The pectin substances had the form of a flocculent white odorless mucilaginous powder soluble in water and practically insoluble in the majority of organic solvents, $[\alpha]_D^{20}$ +140, water, which contained about 1% of nitrogen. The titrimetric method [4] gave the following quantitative characteristics (%): free carboxy groups, K_c – 8.1; methoxylated carboxy groups, K_c – 7.2; degree of esterification – 47; methoxy groups – 5.2. In the

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