POLYPHENOLIC COMPOUNDS OF *Persica vulgaris*

A. A. Sadykov and A. S. Sadykov

UDC 547.972

The results are given of a chemical study of the polyphenol composition of various organs of *Persica vulgaris* Mill. The material of investigation consisted of the dried leaves, flowers, the bark of the roots and of the stems, the fruit, and the skin of the fruit of the peach collected from the experimental plots of the R. R. Shreder Scientific-Research Institute of Horticulture and Viniculture of the Uzbek SSR. Seventeen polyphenols have been isolated, of which 14 have been characterized completely. The amounts of total polyphenols in the skin of the fruit of 10 varieties have been determined. Kaempferol 3-O-β-D-diglucopyranoside and quercetin 3-O-β-D-diglucopyranoside have been isolated from peach leaves for the first time, and (−)-epicatechin gallate from the bark of the stems. The position of the sugar substituent in persicoside has been established: it is hesperitin 5-O-β-D-glucopyranoside.

Of the six species of *Persica* Mill. found in Central Asia [1], the most widespread is the common peach – *Persica vulgaris* (*Prunus persica*). In preceding papers we have discussed the results of a chemical study of the carbohydrates, alcohols, and sterols [2] and, in part, of the flavonoids and catechins [3, 4] and anthocyanins [5-7] of the commonest varieties of peach. In the present communication we give information on the polyphenolic compounds of this plant. The dried leaves, flowers, root bark, stem bark, fruit and skin of the peach collected from experimental plots of the R. R. Shreder Scientific-Research Institute of Horticulture and Viniculture of the Uzbek SSR were investigated.

The raw material was first extracted with chloroform to eliminate resins and pigments. Subsequently, to isolate the polyphenols, extraction was carried out in accordance with schemes which we developed for each type of raw material separately.

The physicochemical characteristics of the polyphenolic compounds isolated from the various organs of the peach are given in Table 1.

The leaves were extracted with an aqueous solution of methanol. After elimination of the methanol, the polyphenols were extracted with ethyl acetate and were precipitated with petroleum ether. A yellow powder of the combined polyphenols was obtained. This combined material was separated into the individual components by column adsorption chromatography on Kapron powder. Elution was carried out with chloroform–methanol (9.5:0.5) with a subsequent increase in the concentration of methanol to a ratio of 4:1. This led to the formation of three zones. The eluates from each respective zone were combined and evaporated, and the residue was recrystallized and subjected to identification tests. In this way we isolated kaempferol 3-O-β-D-glucopyranoside (zone I), kaempferol 3-O-β-D-diglucopyranoside (zone II), and quercetin 3-O-β-D-diglucopyranoside (zone III) [3, 8].

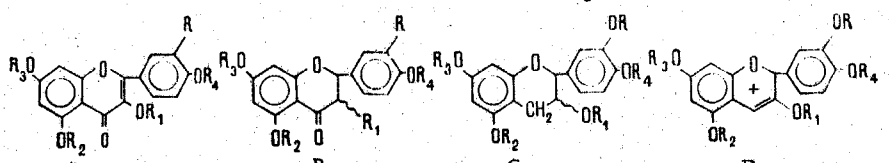
In peach leaves we also found the aglycone kaempferol [9] and its 7-O-glucoside [10]. We are the first to have obtained kaempferol 3-O-β-D-diglucopyranoside and quercetin-O-3-β-D-diglucopyranoside.

The combined polyphenols from the bark of the stems was obtained as described above. To isolate the individual compounds two columns were prepared with the same adsorbent as for the leaves. In one of them the ether-soluble fraction of the total material was separated and in the other the fraction soluble in ethyl acetate. From the first column four individual flavonoids were obtained which were identified as the aglycones persicogenin, naringenin, aromadendrin, and hesperitin [3]. From the second column we obtained two individual glycosides and a mixture of two other substances giving a coloration with the vanillin reagent.

Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent.
Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 316-321, May-June, 1979. Original article submitted October 16, 1978.

0009-3130/79/1503-0273$07.50

TABLE 1. Polyphenolic Compounds Isolated from *Persica vulgaris*



A B C D

Structure	Name	Substituting groups	mp, °C: substance	mp, °C: acetate	$[\alpha]_D^{20}$, deg	λ_{max}, nm	Plant organ	Literature
A	Kaempferol 3-O-β-D-glucoside	R=H; R_1=D-Glc; R_2=R_3=R_4=H	178—180	182	—56 (ethan.)	266,360	Leaves	2,3,5
A	Kaempferol 3-O-β-D-diglucoside	R=H; R_1=D-Glc, Glc; R_2=R_3=R_4=H	214—215	160—163	—56,0 (ethan.)	266,345	Leaves	2,3,5
A	Quercetin 3-O-β-D-diglucoside	R=OH; R_1=D-Glc, Glc: R_2=R_3=R_4=H	205—207	196—197	—80,0 (ethan.)	257,355	Leaves	2,3,5
B	Persicogenin	R=OH; R_1=R_2=H; R_3=R_4=CH_3	164—165	130—132		286,332	Fruit, bark	2,3,5,6
B	Naringenin	R=R_1=R_2=R_3=R_4=H	247—248	124—126		288,315	Stems	2,3,5
B	Aromadendrin	R=H, R_1=OH; R_2=R_3=R_4=H	237—239	119—120	+45,0 water-acetone (1:1)	293,330	Fruit	6
B	Hesperitin	R=OH; R_1=R_2=R_3=H; R_4=CH_3	224—225	143—144	—37,6 (ethan.)	289,330	Stem bark	2,3,5
B	Persicogenin 5-O-β-D-glucoside	R=OH; R_1=H; R_2=D-Glc; R_3=R_4=CH_3	278—280			284,328	Stem bark	2,3,5
B	Naringenin 5-O-β-D-glucoside	R=R_1=H; R_2=D-Glc; R_3=R_4=H	221—225	125—126	—119,5 (ethan.)	225,283	Stem bark	2,3,5
B	Hesperitin 5-O-β-D-glucoside	R=OH; R_1=H; R_2=D-Glc; R_3=H; R_4=CH_3	257—258		—112,8 (ethan.)	287,326	Stem bark	2,3,5
C	(+)-Catechin	R=R_1=R_2=R_3=R_4=H	168—170	132—133	+17,2 water-acetone (1:1)	275	Stem bark	2,4,5
C	(—)-Epicatechin gallate	R=H; R_1= galloyl; R_2=R_3=R_4=H	234—235	119—120	—170 (ethan.)	280	Fruit	2,4,5,6
D	Chrysanthemin	R=H; R_1=D-Glc; R_2=R_3=R_4=H	215—217 (decomp.)			525	Flowers, skin of the fruit	2,4,5,8
D	Chlorogenic acid		204—205		—34 (ethan.)	240,328	Flowers, fruit, skin of the fruit	2,4,5,8

On the basis of the results of hydrolysis, alkaline fusion, and UV and IR spectroscopy, the glycosides were identified as persicogenin 5-O-β-D-glucopyranoside and naringenin 5-O-β-D-glucopyranoside [3, 11].

The residual combined material was chromatographed through a column of silica gel and elution was carried out with peroxide-free moist ether: (+)-catechin and (–)-epicatechin gallate were identified [2].

The flowers were extracted with methanol acidified with hydrochloric acid (1%). The extract, after concentration in a current of nitrogen, was treated with five volumes of dry peroxide-free ether. The precipitate that deposited was separated off and was dried over P_2O_5. The purification and isolation of an individual compound was carried out by adsorption column chromatography on cellulose in the water–acetic acid–hydrochloric acid (82:15:3) system. The colored methocyanin zone was cut out and was eluted with acidified methanol, and the extract was precipited with dry ether. This gave a dark violet substance with a golden tinge. It was identified as chrysanthemin (cyanidin 3-glucoside) [4, 12-14]. This compound has been isolated previously from the peach by other authors, but it was identified only by paper chromatography [15]. Chlorogenic acid was isolated from the flowers and was identified by preparative PC in the butanol–acetic acid–water (4:1:5) system [4, 16].

The combined polyphenols were isolated from the bark of the roots and were separated by the methods described above for the bark of the stems. A glucoside was isolated with mp 257-258°C. Analysis of the PMR spectrum of the aglycone moiety of this compound showed that the signals of the aromatic protons of ring B appeared at 6.7-7.3 ppm. Doublets on 6.7 and 6.45 ppm were assigned to the H-8 and H-6 protons, respectively (J = 2 Hz), and a doublet at 5.32 ppm to the tertiary H-2 protons. A signal at 3.8 ppm corresponded to an OCH_3 group in ring B.

From its physicochemical characteristics, the aglycone was identified as hesperidin [17, 18], and the glycoside as its 5-O-β-D-glucoside [4].

Subsequent elution of the column gave a mixture of catechins, which was separated into its individual compounds by rechromatography on KSK silica gel. (+)-Catechin and (–)-epicatechin gallate were identified [4, 16].

Another three fractions of individual substances were obtained from the first column; from their chemical properties these substances were assigned to the class of proanthocyanidins having a dimeric nature and giving a qualitative reaction with the vanillin reagent. The investigation of these compounds is continuing.

The fruit was extracted successively with ether, ethyl acetate, and methanol. The ethereal extract yielded persicogenin, naringenin, and aromadendrin, the ethyl acetate fraction yielded (+)-catechin and (–)-epicatechin gallate, and the methanol fraction yielded chlorogenic acid [6].

The polyphenols of the skin of the fruit were identified as chrysanthemin, (+)-catechin, and chlorogenic acid [7, 19].

An analysis of the results obtained shows that the polyphenols of the peach consist of various groups of compounds: true flavonoids, flavon-3-cis, flavone-3,4-diols, and hydroxycinnamic acids. The leaves and the bark of the roots and of the stems are rich in flavonoid compounds, and the fruit and flowers are rich in enthocyanins.

Thus, peach wastes may be sources for the isolation of these substances for their use in pharmacy [20, 22] and the food industry as vitaminized additives and dyes [7].

EXPERIMENTAL

The NMR and PMR spectra were recorded on an XL-100 spectrometer* (Varian). As the samples we used 8-10% solutions of the compounds under investigation in deuterochloroform and in CCl_4 (with HMDS as internal standard). The mass spectra were recorded on a Varian XL-100 instrument. The melting points were determined on a Kofler block and the optical activities on a CM circular polarimeter. The purities of the substances were checked by PC on "M" ["slow"] paper in the following solvent systems: 1) butan-1-ol–acetic acid–water (4:1:5); 2) benzene–acetic acid–water (125:72:3); and 3) butan-1-ol–acetic acid–water (40:12.5:29).

*As in Russian Original – Publisher.

In the separation of the individual substances we used the solvent systems 4) chloroform–methanol (9.5:0.5 and 8:2) and 5) water–acetic acid–hydrochloric acid (82:15:3). The UV spectra were recorded on a SF-4A spectrophotometer and the IR spectra on UR-10 and IKS-22 spectrometers. The analyses of all the compounds corresponded to the calculated figures.

Flavonoids from the Leaves. The comminuted leaves (1 kg) were treated with chloroform to eliminate resins, and the flavonoids were extracted with aqueous methanol. The combined methanolic extracts were evaporated in vacuum and the residue was then extracted with ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and were evaporated to small volume under reduced pressure in a current of nitrogen at 40-45°C. Five volumes of dry petroleum ether were added to the concentrated extract. A flocculant yellow precipitate deposited, which was washed with dry chloroform and dried in a vacuum desiccator. Yield 39 g.

Kapron powder (200 g) was mixed with chloroform and introduced into a column (4.5×90 cm), and the adsorbent was compacted under vacuum. The total polyphenols (10 g) were dissolved in 6 ml of acetone, and the solution was carefully mixed with 5 g of Kapron and the mixture was dried at room temperature to eliminate solvent. Then it was deposited on the prepared column. The column was washed with system 4 with a subsequent increase in the concentration of methanol. This produced a clear separation of the column into three zones. Zone I contained kaempferol 3-O-β-D-glucopyranoside, R_f 0.70 (in system 1); light yellow acicular crystals with mp 178-180°C.

Acid Hydrolysis. The glucoside (0.05 g) was hydrolyzed with 7% HCl for 5 h. Kaempferol with R_f 0.83 (in system 1) and mp 276°C was isolated from the hydrolysate. Glucose with R_f 0.50 (in system 1) was detected.

Acetylation. The substance (0.05 g) was heated on the water bath with acetic anhydride in the presence of sodium acetate for two hours, and then the reaction mixture was diluted with ice water. The precipitate that deposited was filtered off, washed with water to neutrality, and recrystallized from ethanol. White acicular crystals with mp 182°C deposited.

Alkaline Cleavage of Kaempferol. A mixture of 0.06 g of the aglycone, 0.5 g of caustic potash, and 1 ml of water as heated on a sand bath at 200-230°C for 15 min. After cooling, the reaction mixture was acidified with sulfuric acid and was extracted several times with ether. The ether was distilled off and the residue was analyzed by chromatography in system 3.

Revealing agent: an equal mixture of a 1% aqueous solution of ferric chloride and potassium ferricyanide and a 1% solution of vanillin in concentrated sulfuric acid. The red-orange spot of phloroglucinol with R_f 0.75 and the blue spot of p-hydroxybenzoic acid with R_f 0.88, identical with the spots of authentic samples, were detected.

Zone II contained kaempferol 3-O-β-D-diglucopyranoside, R_f 0.43 (in system 1); pale yellow acicular crystals with mp 214-215°C.

Acid hydrolysis gave the aglycone kaempferol with a yield of 46.7%, and glucose. The ratio of kaempferol and glucose was 1:2. λ_{max}: C_2H_5OH – 2.87, 326; CH_3COONa – 295, 394; $AlCl_3$ – 289, 331; $CH_3COONa + H_3BO_3$ – 312, 351 nm.

Zone III contained quercetin 3-O-di-β-D-glucopyranoside, R_f 0.37 (in system 1); light yellow acicular crystals with mp 205-207°C. Kaempferol 3-O-di-β-D-glucopyranoside and quercetin 3-O-di-β-D-glucopyranoside were identified by the scheme described above.

Polyphenols from the Bark of the Stems. The bark of the stems (1 kg) was treated by the method described above. This gave 133.6 g of total material. For separation, 20 g of the combined polyphenols was treated with diethyl ether. The ethereal fraction was concentrated and was carefully mixed with Kapron powder, and the mixture was dried at room temperature to eliminate the solvent. A Kapron column (4.5×70 cm) was prepared by the method described above and the Kapron powder with the total polyphenols absorbed on it was transferred to the column. The column was washed with system 4. This gave eluate fractions I-IV.

Fraction I yielded persicogenin in the form of colorless acicular crystals with mp 164-165°C, R_f 0.92 (system 1); acetyl derivative, mp 130-132°C. Fraction II yielded naringenin in the form of colorless crystals with mp 247-248°C, R_f 0.92. Fraction III yielded aromadendrin in the form of colorless acicular crystals with mp 237-239°C, R_f 0.87. Fraction IV yielded hesperitin in the form of colorless acicular crystals with mp 224-225°C, R_f 0.90. The acetylation and alkaline fusion of these compounds were performed by the methods described above.

The residual combined material after the separation of the ether-soluble fraction was treated with ethyl acetate and the extract was separated on a chromatographic column prepared as described above. This gave fractions V-VII. Fraction V yielded persicogenin 5-O-β-C-glucopyranoside in the form of yellowish crystals with mp 221-225°C. When the chromatogram was treated with sulfanilic acid, it gave an orange-brown spot. The acidic and enzymatic hydrolyses and alkaline fusions of the compounds mentioned were performed by the usual methods.

Fraction VII (total catechins) was passed through a column of silica gel. The column was washed with peroxide-free moist diethyl ether. The separation of the total catechins was monitored by paper chromatography. As a result, two fractions were obtained. The eluates were evaporated under reduced pressure in a current of nitrogen and the residues were dissolved in small amounts of hot water from which, on standing in the cold, colorless acicular crystals of (+)-catechin with mp 168-170°C and of (–)-epicatechin gallate with mp 234-235°C deposited.

Polyphenols of Peach Blossoms. A mixture of 100 g of peach blossoms and 0.6 liter of chloroform was allowed to stand for a day and was extracted three times to eliminate fatty and waxy substances. After extraction, the raw material was dried and was extracted with methanol containing 1% of hydrochloric acid (3×0.5 liter) at room temperature. The extract was concentrated under reduced pressure in a current of nitrogen at 35-40°C to small volume and was left in the refrigerator for 16 h. The white amorphous precipitate that deposited was separated off, and the filtrate was treated with three volumes of dry ether (peroxide-free). A red flocculant precipitate deposited, which was immediately filtered off, washed with dry acetone, and rapidly dried over P_2O_5. This gave 1.3 g of anthocyanins (1.3% of the weight of the air-dry blossoms).

The residue was dissolved in system 5 and passed through a column (60×3 cm) filled with cellulose powder. The column was washed with the same mixture until a band appeared distinctly in the form of a ring. The band was cut out and was eluted with methanol containing 0.1% of hydrochloric acid. After concentration of the solution, it was treated with 10 volumes of dry ethyl ether. The precipitate that deposited was filtered off, washed, and dried. This gave chrysanthemin as a dark violet substance with a golden tinge having mp 215-217°C (decomp.). Chlorogenic acid was isolated preparatively (PC, system 1) and identified (mp 204-205°C).

Polyphenols of the Bark of the Roots. The bark of the roots (2 kg) was treated by the method described above, which gave the total polyphenols (412.6 g; 20.63% on the air-dry weight). Separation was carried out as described above, leading to the isolation of hesperitin 5-O-β-D-glucoside, (+)-catechin and (–)-epicatechin gallate, and three dimeric proanthocyanidins.

Polyphenols of the Fruit. Peaches (1 kg) were extracted with ether, and then successively with ethyl acetate and methanol. The first two fractions yielded 2.5 and 16 g of combined polyphenols, respectively. The methanolic fraction yielded 270 g of a resinous brownish substance.

Separation of the combined flavonoids from the ethereal fraction on Kapron powder yielded the aglycones persicogenin with mp 164-165°C, naringenin with mp 247-248°C, and aromadendrin with mp 237-239°C.

From the total ethyl-acetate-soluble material (+)-catechin and (–)-epicatechin gallate were isolated by means of a silica gel column. Chlorogenic acid was obtained by preparative PC (Table 1).

Polyphenols of the Skin of the Fruit. Samples (100 g) of the skin of the fruit of 10 varieties of peach were extracted by steeping with acidified methanol. Chrysanthemin, (+)-catechin, and chlorogenic acid were isolated by the scheme described above.

SUMMARY

From various organs of *Persica vulgaris* Mill. a total of 17 individual polyphenolic compounds have been obtained, of which 14 have been identified.

Kaempferol 3-O-di-β-D-glucopyranoside and quercetin 3-O-di-β-D-glucopyranoside have been isolated from peach leaves for the first time, and (–)-epicatechin gallate from the bark of the stems.

The position of the sugar substituent in persicoside has been established: it is hesperitin 5-O-β-D-glucopyranoside.

LITERATURE CITED

1. Flora of Uzbekistan [in Russian], Tashkent, Vol. III (1955), p. 379.
2. A. A. Sadykov et al., Dep. VINITI, No. 4448-76.
3. A. A. Sadykov, Kh. I. Isaev, and A. I. Ismailov, Khim. Prir. Soedin., 94 (1975).
4. A. A. Sadykov, Kh. I. Isaev, and A. I. Ismailov, Khim. Prir. Soedin., 281 (1975).
5. A. A. Sadykov, Khim. Prir. Soedin., 107 (1977).
6. A. A. Sadykov, Khim. Prir. Soedin., 133 (1977).
7. A. A. Sadykov, Kh. I. Isaev, and T. A. Vdovtseva, Khim. Prir. Soedin., 405 (1978).
8. I. I. Samkoish et al., Khim. Prir. Soedin., 439 (1969).
9. L. Cronenberger, Compt. Rend., 249, No. 25, 2866 (1959).
10. H. Pacheco, Compt. Rend., 251, 1653 (1960).
11. P. N. Corgan, Proc. Am. Soc. Hortic. Sci., 86, 129 (1965).
12. K. Mackaji, M. Sunagawa, and H. Imai, Agric. Biol. Chem., 27, No. 3, 165 (1965).
13. I. M. Hais and K. Macek, Paper Chromatography, 3rd ed., Academic Press, New York (1963).
14. C. C. Craft, Proc. Am. Soc. Hortic. Sci., 78, 119 (1961).
15. C. L. Hsia, B. S. Luh, and C. O. Chichester, J. Food Sci., 30, No. 1, 5 (1965).
16. M. N. Zaprometov, The Biochemistry of the Catechins [in Russian], Moscow (1964).
17. W. Rahman and S. P. Bhatnacer, Aust. J. Chem., 21, 539 (1968).
18. H. Pacheco, Bull. Soc. Chim. Biol., 39, 971 (1954).
19. Z. B. Rakhimkhanov, A. K. Karimdzhanov, A. I. Ismailov, and A. S. Sadykov, Khim. Prir. Soedin., 190 (1968).
20. F. R. Khalikova and A. A. Sadykov, Abstracts of the Fourth Republican Conference of the Central Scientific-Research Laboratory of the Intermediate Colleges of Uzbekistan [in Russian], Tashkent, Vol. IV (1978), p. 128.
21. L. S. Evseenko, in: Phenolic Compounds and Their Biological Functions [in Russian] (1968), p. 348.
22. V. A. Baraboi, The Biological Action of the Phenolic Compounds of Plants [in Russian], Kiev (1976), p. 240.

A STUDY OF THE CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *Ledum palustre*

N. S. Mikhailova, K. S. Rybalko, and V. I. Sheichenko

UDC 547.597+547.913+638.88

Eight substances have been isolated by preparative chromatography on silica gel from the essential oil of *Ledum palustre* L., collected in the Kostroma oblast in August. Six of them have been described previously for this essential oil (myrcene, palustrol, ledol, allocaromadendrene, 6-methyl-2-methyleneocta-5,7-dien-3-ol, and cyclocolorenone) and two are new substances not described in the literature: a colorless liquid with a pleasant smell having the composition $C_{10}H_{14}O_2$, mp 108-111°C/4 mm Hg, n_D^{20} 1.4925, d_{20}^{20} 1.002, $[\alpha]_D \pm 0°C$, which has been called lepalol, and a colorless liquid with a pleasant smell having the composition $C_{10}H_{12}O$, bp 91-93°C/4 mm Hg, n_D^{20} 1.5175, d_{20}^{20} 0.9444, $[\alpha]_D \pm 0°C$, which have been proposed for lepalin and lepalol on the basis of their IR, UV, and NMR spectra. The physical constants of all the substances isolated and the characteristics of their IR, NMR, and mass spectra are given.

The chemical composition of the essential oil of crystal tea ledum *Ledum palustre* L. varies considerably. Some authors connect this with the growth site [1-3] and others with botanical varieties [4, 5].

The composition of the essential oil of crystal tea ledum growing in the USSR has been studied by N. P. Kir'yalov. He established that the essential oil of crystal tea ledum from

All-Union Scientific-Research Institute of Medicinal Plants, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 322-325, May-June, 1979. Original article submitted January 30, 1979.

 0009-3130/79/1503-0278$07.50