UDC 547.917

FLAVONOIDS OF PHELLODENDRON SACHALINENSE AND PH. AMURENSE

O. I. Shevchuk, N. P. Maksyutina, and V. I. Litvinenko

Khimiya Prirodnykh Soedinenii, Vol. 4, No. 2, pp. 77-82, 1968

It is known that the leaves of Phellodendron japonicum Max. (Japanese cork tree) and Ph. amurense Rupr. (Amur cork tree) contain a considerable amount of flavonoid compounds [1-3]. We have studied the flavonoid composition of the leaves of the Sakhalin cork tree (Ph. sachalinense (F. Schm.) Sarg) in comparison with the flavonoids of the Amur cork tree. The plants studied were grown in the Botanical Gardens of AS UkSSR, Kiev. It was found that both species of cork tree contained about 10% of total flavonoid compounds representing more than ten components.

The combined flavonoids were separated into fractions, and three individual compounds were isolated. One of them, from its physicochemical properties, was identical with hyperin [3], while the other two are probably new, since they are distinguished by certain properties from fellamurin [1], amurensin [1], and phellodendroside [2] (see table) which have been isolated previously from the Amur and Japanese cork trees.

The substances, which we have called phelloside and dihydrophelloside, give a positive cyanidin reaction and may therefore be assigned to the flavonoid group. The bright red pigments obtained in the cyanidin reaction are soluble in water. Fehling's solution is reduced by solutions of these compounds only after acid hydrolysis, which is characteristic for glycosides. On borohydride reduction a red pigment is formed from dihydrophelloside, as in the case of flavanone derivatives [4]. With zirconyl nitrate, solutions of both compounds are colored yellow, but the addition of citric acid decolorizes the dihydrophelloside solution [5].

The results of spectral studies in the UV region (see table), together with the qualitative reactions, show that phelloside (I) is a flavonol and dihydrophelloside (II) a flavanone [6]. In the presence of sodium acetate, the spectrum of phelloside shows a considerable hypsochromic shift. This phenomenon is characteristic of flavonols with a free 3,4'-dihydroxy grouping [6]. In the spectrum of dihydrophelloside, instead of the expected bathochromic shift of the long-wave maximum by 100-110 mμ that is observed in flavanones with free 4'- and substituted 7-hydroxy groups [6, 7], a shift of only 30 mμ was found. In the spectrum of dihydrophelloside, zirconyl nitrate caused a bathochromic shift of 60 mμ which disappeared when citric acid was added, and in that of phelloside a shift of 95 mμ which merely fell to 50 mμ. Thus, it was shown that phelloside has free hydroxy groups in positions 3 and 5 and dihydrophelloside a free hydroxy group only in position 5 [8].

On oxidation with gaseous oxygen in aqueous solution, dihydrophelloside is slowly converted into phelloside. A similar but faster conversion takes place when it is oxidized with hydrogen peroxide in an alkaline medium [1, 2]. Consequently, the substance under investigation, provisionally described as a flavanone, is converted by oxidation into a flavonol. As is well known, the same properties are possessed by the 3-hydroxyflavanones of the 2H:3H-trans series [9]. Consequently, dihydrophelloside is probably a flavanonol glycoside.

The aglycones of phelloside and dihydrophelloside obtained after acid and enzymatic hydrolysis contained the same free hydroxy groups as the initial glycosides and could not be identified with known common aglycones. However, on the basis of literature information on the flavonoids of the cork tree [1, 2], we assumed that the glycosides studied were derivatives of alkylated compounds.

In order to eliminate a possible alkyl substituent, we treated the aglycone of phelloside with hydriodic acid in phenol and acetic anhydride, and from the reaction products we isolated kaempferol (VIII) [10]. Alkaline cleavage of the aglycone and steam distillation of the products gave isovaleric acid (VII) [1, 11]. These results permitted a comparison of the aglycones of phelloside and dihydrophelloside with β-anhydronoricaritin (VI) and phellamuretin (V) and the establishment of their respective identities.

A study of the spectral properties of the glycosides and their aglycones in the UV region showed, from the intensity of their absorption ($E_{1\,cm}^{1\%}$), that the glycosides must each contain two molecules of D-glucose [12]. This was confirmed by determining the proportion of aglycones (48-50%) after complete acid hydrolysis of the substances obtained.

We obtained additional information on the structure of the glycosides in a study of the products of stepwise acid hydrolysis. Phelloside formed two monoglucosides, which were isolated by partition chromatography on a column of hydrocellulose. One of the monosides was identical in its physicochemical properties with amurensin (III), which contains a carbohydrate substituent in position 7 [1]. The second had a free 7-hydroxy group and its carbohydrate substituent was probably attached to an alkyl substituent in the γ position. A knowledge of the optical rotation of this glycoside, −38.0°, also indicates the alkyl nature of the glycoside, which has been named noricariside-1 (IV), since amurensin has an optical rotation of −66.0°. Furthermore, if the γ-hydroxy group in the isopentyl substituent were not substituted by a sugar, under the conditions of acid hydrolysis the free 7-hydroxy group would undergo dehydration with the appearance of a

Physicochemical Properties of the Flavonoids of Sakhalin and Amur Cork Trees

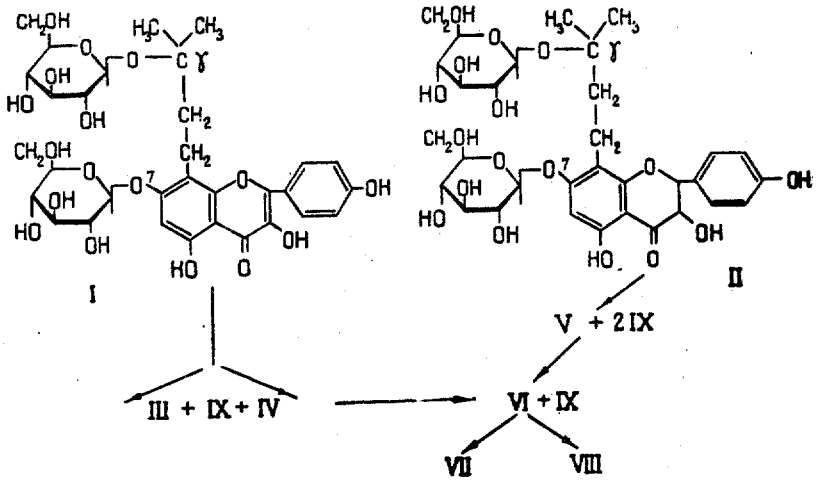
Flavonoids	Mp, °C	Optical activity, deg.	Spectral properties of flavonoids in the UV region λ, mμ										$E^{1\%}_{1\ cm}$	Proportion of aglycone %
			absorption bands	ethanolic solution		ethanolic solution + sodium acetate		ethanolic solution + sodium ethoxide		ethanolic solution + zirconyl nitrate		ethanolic solution + zirconyl nitrate and citric acid		
				λ	log ε	λ	Δλ	λ	Δλ	λ	Δλ	λ	Δλ	
Phelloside (B)	282—284	—90,0	I	375	4,21	375	0	335	—40	470	+95	425	+50	306	50,8
			II	270	4,25	272	+2	270	0	263	—7	270	0		
Monoglucoside B-1[a] (amurensin)	290—291	—66,0	I	365	4,25	365	0	310	—55	455	+90	415	+50	475	66,3
			II	265	4,30	270	+5	245	—20	270	+5	265	0		
Monoglucoside B-2[b] (noricariside-1)	293—294	—38,0	I	375	4,27	415	+40	305	—70	455	+80	430	+55	487	66,5
			II	270	4,32	260	—10	250	—20	280	+10	270	0		
Aglycone (B)[c]	302—305	—	I	375	4,29	375	0	335	—40	455	+80	430	+55	841	—
			II	270	4,38	275	+5	275	+5	280	+10	270	0		
Dihydrophelloside (A)[d]	152—153	—	I	345	3,53	345						345			
			II	290	4,26	290	0	375	+30	405	+60	290	0	282	51.0
				225	4,65	225	0	225	0	225	0	225	0	—	—
Aglycone (A)[e] (phellamuretin)	218—220	—	I	345	3.53	345	0	375	+30	405	+60	345	—	—	—
			II	290	4,25	290						290	0	856	—
				225	4,67	225	0	225	0	225	0	225	0		

[a]Amurensin [1]-mp 290° C; λ_{max} in the UV spectrum 377 and 270 mμ (log ε 4.23 and 4.27).
[b]Noricariside [1, 11]-mp 271-272° C, $[\alpha]_D$ -15.21 (pyridine).
[c]Nor-β-anhydroicaritin [1]-mp 305° C; λ_{max} in the UV spectrum 365 and 271 mμ (log ε 4.32 and 4.38).
[d]Phellodendroside [2]-mp 154-156° C; λ_{max} in the UV spectrum 220 and 298 mμ.
[e]Phellamuretin [1, 2]-mp 220° C; λ_{max} in the UV spectrum 300 mμ (log ε 4.28).

pyran ring, as in the case of phellamuretin and β-anydronoricaritin [1, 11], formed on the complete cleavage of the glycosides.

The results of enzymatic cleavage of the monoglucosides and also a comparison of the molecular rotations with the corresponding phenyl and methyl glucosides give grounds for assuming that the D-glucose (IX) is present in the pyranose form and the glycosidic linkage with the aglycone has the β configuration.

Thus, phelloside and dihydrophelloside are 7,γ-di(O-β-D-glucopyranosides). Their structural formulas and chemical reactions can be represented in the following way:



Experimental

The chromatographic analysis of the flavonoids and sugars was carried out on Goznak-85 paper in the following systems: 1) 15% acetic acid; 2) 30% acetic acid; 3) 1-butanol–acetic acid–water (4:1:5); 4) benzene–ethyl acetate–acetic acid–formamide (24.5:73.5:2:1); 5) 60% acetic acid; 6) ethanol–water–25% ammonia (8:1:1). The spectral studies in the UV region were carried out on an SF-4A spectrophotometer and those in the IR region on a UR-10 spectrometer. The optical activities were measured on an SPU-3 spectropolarimeter.

Isolation of the flavonoids. One kilogram of the leaves of the Sakhalin cork tree or the Amur cork tree was extracted with methanol (10 *l*). The extract was evaporated in vacuum to small volume, diluted with water to 0.5 *l* and freed from chlorophyll with chloroform. The flavonoids were extracted from the aqueous solution with ethyl acetate, and after evaporation of the extract the combined flavonoids were again brought into aqueous solution. The substances separated into two fractions. The aqueous solution contained mainly dihydrophelloside and the precipitate phelloside. The phelloside formed yellow needle-like crystals with mp 282–284° C (from 50% ethanol).

Found, %: C 55.07; 54.98; H 5.75; 5.70. Calculated for $C_{32}H_{40}O_{17}$, %: C 55.01; H 5.73.

On cooling, the aqueous solution deposited dihydrophelloside in the form of white needle-like crystals with mp 150–152° C (from water).

Found, %: C 55.67; 56.59; H 6.23; 6.15. Calculated for $C_{32}H_{42}O_{17}$, %: C 56.63; H 6.19.

Oxidation of dihydrophelloside. A. Oxidation with oxygen. 2.0 g of dihydrophelloside was dissolved in 200 ml of boiling water, and at a solution temperature of 50° C oxygen was passed through at the rate of 60 bubbles per min for a week. The phelloside was produced in the form of a yellow crystalline precipitate.

B. Oxidation with hydrogen peroxide. A solution of 2.0 g of dihydrophelloside in 40 ml of methanol was treated with 5 ml of 10% caustic potash and 1 ml of perhydrol, and the mixture was left for a day at 4° C. Then it was diluted with water to 150 ml and neutralized with acid. A precipitate of phelloside deposited.

Complete acid hydrolysis of the glycosides. In drops, 20 ml of concentrated sulfuric acid was added to a suspension of 1.0 g of phelloside in 20 ml of water. After 20 min, the solution was cooled and neutralized with 10% caustic potash. This gave a precipitate of the aglycone (0.508 g) with mp 303–305° C (from ethanol).

Found, %: C 67.72; 67.81; H 5.12; 5.17. Calculated for $C_{20}H_{20}O_6$, %: C 67.79; H 5.08.

After evaporation to dryness and extraction with ethanol, the aqueous fraction was analyzed by paper chromatography, and glucose was found. The hydrolysis of dihydrophelloside (1.0 g) under similar conditions gave the aglycone (0.510 g) in the form of white needle-like crystals with mp 218–220° C (from water).

Found, %: C 67.47; 67.51; H 5.59; 5.63. Calculated for $C_{20}H_{20}O_6$, %: C 67.41; H 5.61.

After chromatographic analysis, the carbohydrates of the hydrolyzate were identified as D-glucose.

Enzymatic cleavage of the glycosides. A solution of 1.0 g of phelloside in 1 ml of dimethylformamide was diluted with water to 50 ml, 0.2 g of emulsin was added, and the mixture was left to hydrolyze in the thermostat at 36° C for 24 hr. Then ethanol was added to a volume of 150 ml and the mixture was heated on the water bath for 30 min. The precipitate that deposited was filtered off, and the ethanol was driven off from the filtrate. On cooling, a yellow crystalline precipitate of the aglycone deposited with mp 304–305° C (from ethanol). The carbohydrates were identified as D-glucose. Dihydrophelloside was hydrolyzed under similar conditions. This gave an aglycone with mp 219–220° C and D-glucose.

Stepwise acid hydrolysis of phelloside. A solution of 2.0 g of phelloside in 200 ml of 50% ethanol containing 8% of sulfuric acid was hydrolyzed by being heated in the water bath, the cleavage products being analyzed every 5 min. The maximum amount of intermediate products was formed after 30 min. At this stage the hydrolysis was stopped, and the solution was neutralized with barium carbonate and evaporated to small bulk. The mixture of flavonoids was separated on a column of regenerated cellulose with elution by means of 30% acetic acid. After well-separated zones had formed on the column, elution was stopped and the column was divided according to the zones. Each portion of sorbent was eluted with cold water to neutrality and the flavonoids were extracted with 70% ethanol. The extracts were analyzed by paper chromatography in several systems of solvents. The first portion was found to contain the pure starting material (R_f 0.31, 0.54, 0.73), the third portion noricariside-1 composition $C_{26}H_{30}O_{12}$, mp 293–294° C (from ethanol), and R_f 0.25, 0.41, 0.64, and the fifth amurensin $C_{26}H_{30}O_{12}$, mp 290–291° C (from 50% ethanol, R_f 0.15, 0.27, and 0.54 in systems 1, 2, and 5, respectively).

Dealkylation of the aglycone of phelloside. A mixture of 0.2 g of the aglycone with 4 ml of hydriodic acid (sp. gr. 1.7), and 3.2 ml of liquid phenol was heated at a gentle boil for 10 hr. After cooling, the reaction mixture was poured into 60 ml of 20% sodium thiosulfate solution. The precipitate that deposited was filtered off and dissolved in ethanol, the insoluble part being separated off. The filtrate diluted with water yielded kaempferol $C_{15}H_{10}O_6$ with mp 278–279° C.

Alkaline cleavage of the aglycone of phelloside. A solution of 1.0 g of the aglycone in 30 g of fused caustic potash and 1 ml of water was kept in the fused state for 10 min. Then it was cooled and the mixture was dissolved in 200 ml of water and neutralized with 10% hydrochloric acid to pH 3.0. The steam-distilled cleavage products were found by paper chromatography with reference samples in systems 3 and 6 (with Bromothymol Blue as the staining agent) to contain isovaleric acid.

Summary

1. About ten substances of a flavonoid nature have been found in the leaves of Phellodendron sachalinense (F. Schm.) Sarg. and Ph. amurense Rupr.

2. The two species of cork tree each contain two new flavonoids, which have been called phelloside and dihydrophelloside. The results of chemical and spectroscopic studies have permitted phelloside to be characterized as the 7,γ-di-O-β-D-glucopyranoside of noricaritin and dihydrophelloside as the 7,γ-di-O-β-D-glucopyranoside of dihydronoricaritin, the base of which contains kaempferol and aromadendrin.

3. Amurensin (the 7-β-D-glucopyranoside of noricaritin) and icariside-1 (the λ-O-β-D-glucopyranoside of noricaritin) have been isolated as intermediates in the stepwise hydrolysis of phelloside; the latter has not been previously found in the Amur and Japanese cork trees.

REFERENCES

1. M. Hasegawa, T. Shirato, J. Amer. Chem. Soc., 75, 5507, 1953.
2. T. Bodalski and E. Lamer, Dissert. Pharmaceut., 15, 319, 1963.
3. T. Bodalski and E. Lamer, Dissert. Pharmaceut., 16, 67, 1964.
4. R. M. Horowitz, J. Org. Chem., 22, 1733, 1957.
5. L. Hörhammer and K. H. Müller, Arch. Pharm., 287, 310, 1954.
6. L. Hurd, Spectral Properties of Flavonoid Compounds, in The Chemistry of Flavonoid Compounds, ed. T. A. Geissman, Pergamon Press, N. Y., 107, 1962.
7. V. I. Litvinenko, N. P. Maksyutina, and D. G. Kolesnikov, ZhOKh, 33, 4014, 1963.
8. V. I. Litvinenko and N. P. Maksyutina, KhPS [Chemistry of Natural Compounds], 420, 1965.
9. J. W. Clark-Lewis, Rev. Pure and Appl. Chem., 12, 96, 1962.
10. M. M. Pashchenko, G. P. Pivnenko, and V. I. Litvinenko, Farm. Zh. (Kiev), 21, no. 1, 44, 1966.

11. S. Akai, J. Pharm. Soc., Japan, 55, 112, 1935.
12. V. I. Litvinenko, Rastitel'nye Resursy, 2, no. 4, 65, 1966.

7 December 1966

Khar'kov Chemical and Pharmaceutical Scientific Research Institute,
Kiev Institute for the Improvement of Drugs