# A CHEMICAL STUDY OF THE ROOTS OF Paeonia anomala

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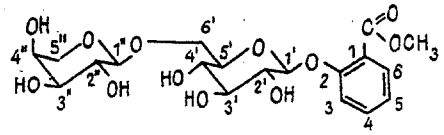
The new phenolic glycoside paeonovicinoside (methyl salicylate 6'-α-L-arabinopyranosyl-β-D-glucopyranoside), and also paeoniflorin, β-sitosterol, benzoic acid, and gallic acid and its methyl ester have been isolated from the roots of Paeonia anomala.

A tincture of the herbage and roots of Ural peony Paeonia anomala L. (family Paeoniaceae) is used in medical practice as a sedative [1].

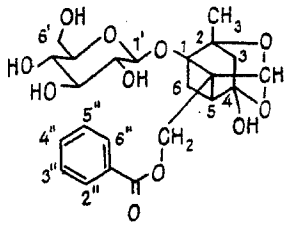
In the literature, cineole, β-sitosterol, β-amyrin, benzoic acid, salicyclic acid, methyl salicylate, salicin, gallic acid, methyl gallate, paeonolide, and paeoniflorin have been described in the literature for Ural peony roots [1, 2].

The aim of the present work was to study the chemical composition of an industrial batch of Ural peony rhizomes.

In the course of the chromatography of an aqueous alcoholic extract of Ural peony roots on silica gel, polyamide, and Sephadex LH-20, six individual compounds were obtained, compound (I), paeonoflorin (II), β-sitosterol (III), benzoic acid (IV), gallic acid (V), and ethyl gallate (VI). Compound (I) was new, and we have called it paeonovicinoside.



Paeonovicianoside



Paeoniflorin

Paeonovicinoside is readily (in the course of 5 min) cleaved under the action of 2% HCl with the formation of glucose, arabinose, and methyl salicylate (a liquid with $M^+$ 152 having a characteristic odor).

The attachment of the carbohydrate fragment to the aromatic OH group followed from the fact that the aglycon obtained was revealed by diazotized sulfanilic acid (DSA) on Silufol UV 254 plates in the form of a yellow spot, while the initial substance did not react with DSA. Furthermore, the presence of a free phenolic OH group in the aglycon was confirmed by the qualitative reaction with $FeCl_3$ solution (violet coloration).

We established the structure of the biose in the molecule of compound (I) by means of a comparison of the $^1H$ NMR spectra of the initial glycoside and its hexaacetate with the $^1H$ NMR spectra of rosavin (cinnamyl alcohol vicianoside), its hexaacetate [3], and the acetates of other arabinosides [4].

When compound (I) was acetylated, the signals of the gem-acyl methine protons (H-2', -3', -4', -2", -3", -4") underwent the greatest paramagnetic shift (4.9-5.3 ppm region). The position of the signals of the anomeric protons of the glucose (H-1') and arabinose (H-1") residues scarcely changed. The signals of five protons remained in a stronger field (3.5-4.0 ppm): H-5' and 2H-6' of the glucose residue and the readily identified [4] signals

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of the two protons at C-5" of the arabinose residue. If the arabinose residue had been attached not to the methylene (6') but to one of the methine OH groups (2', 3', or 4') of the glucopyranose residue, the signals of only four protons in the diagnostic region of 3.5-4.0 ppm should have been expected for the acetate of (I) [3, 4].

Thus, paeonovicianoside (I) has the structure of methyl salicylate 2-O-(6'-O-α-L-arabinopyranosyl-β-d-glucopyranoside).

It must be mentioned that Polish scientists [2] have isolated from Ural peony roots an arabinoglucoside of 2-hydroxy-4-methoxyacetophenone (paeonolide), which has been described previously for the roots of Paeonia suffruticosa [5, 6]. Our experiments did not confirm the Polish results [2] on the presence of paeonoside in the plant under investigation.

We identified compound (II) as paeoniflorin, which has been described previously for the roots of Paeonia albiflora [7-9] and P. anomala [2]. This compound was characterized previously only in the form of various derivatives [7, 8]. We are the first to have recorded its complete $^1$H NMR spectrum (see Experimental), which corresponds to the structure of paeonoflorin (II) established previously [7-9].

Compound (II) was readily hydrolyzed by β-glucosidase with the formation of the aglycon and glucose. The mass spectrum of glycoside (II) showed intense peaks only of benzoic acid fragments (m/z 122, 105, 77).

Ethyl gallate (VI) has not been detected previously in the plant under investigation, and the possibility of its formation as an artefact of gallic acid in the course of the extraction of the raw material with aqueous ethanol cannot be excluded.

## EXPERIMENTAL

$^1$H NMR spectra were obtained on a Gemini-200 (200 MHz) instrument (δ scale 0 – TMS), mass spectra on a Varian CH-8 instrument at 70 eV, and UV spectra on a Specord M40 spectrometer. Angles of rotation were obtained on a Polomat A polarimeter at 546 nm and were recalculated to 589.3 nm.

Chromatographic monitoring was effected on Silufol UV 254 plates in the chloroform–methanol–water (26:14:3), chloroform–methanol (6:1), and benzene–acetone (4:1 systems) and by PC (identification of the sugars) in the ethyl acetate–n-propanol–water (7:2:1) system.

In the case of TLC on Silufol UV 254 the substances were detected by diazotized sulfanilic acid in 10% sodium carbonate solution.

Isolation of the Substances. The ground air-dry roots of ural peony (an industrial batch of raw material from the Batumi Pharmaceutical Chemicals Factory) (300 g) were exhaustively extracted with aqueous ethanol with heating. The extracts were evaporated in vacuum in a syrupy residue, which was dried on silica gel and chromatographed on a column of silica gel L 100/250 using gradient elution with chloroform–methanol mixtures.

The fractions containing paeonovicianoside were crystallized from ethanol. This gave compound (I) containing gallic acid (IV) as an impurity. The compound (I) was purified by filtering an aqueous solution through Woelm polyamide followed by crystallization of the evaporated filtrate from ethanol. When the polyamide was then washed with ethanol, gallic acid (IV) was isolated.

The fractions containing paeoniflorin (II) were chromatographed repeatedly on various sorbents: polyamide (water), silica gel (chloroform–methanol), polyamide (chloroform–methanol), and Sephadex (chloroform–methanol). This gave paeoniflorin (II) in the form of a white amorphous powder.

Chromatography of the nonpolar fractions yielded β-sitosterol (III), benzoic acid (VI), and ethyl gallate (V).

Paeonovicianoside (I), yield 0.3%. White lustrous tabular crystals with the composition $C_{19}H_{26}O_{12}$, mp 166.5-168°C (ethanol), $[\alpha]_D^{25}$ –28.93° (c 1.62, water), $\lambda_{max}^{EtOH}$ 232, 288 nm. $^1$H NMR spectrum ($D_2O$, 200 MHz), δ: 7.69 (1H, dd, 8.6 and 2 Hz, H-3), 7.50 (1H, td, 8.6 and 2 Hz, H-4), 7.25 (1H, d, 8.6 Hz, H-6), 7.10 (1H, t, 8.6 Hz, H-5), 5.08 (1H, d, 7.3 Hz, H-1'), 4.22 (1H, d, 7.3 Hz, H-1"), 4.04 (1H, d, 11 Hz, H-6'), 3.80 (3H, s, $CH_3O$), 3.8-3.3 (m, 10H of a biose). Mass-spectrum, m/z (%): 152 (M+ of the aglycon, $C_8H_8O_3$) (72), 121 (55 ), 120 (100), 92 (61).

Hexaacetate of (I). White acicular crystals with mp 211-213°C (ethanol), $[\alpha]_D^{25}$ -36.18° (c 1.04; chlf). $^1$H NMR spectrum ($CDCl_3$, 200 MHz), δ: 7.75 (dd, H-3), 7.56 (td, H-4), 7.10 (m, H-5,6), 5.3-4.9 (m, 7H: H-1', 2', 3', 4', 2", 3", 4"), 4.43 (1H, d, 7.1 Hz, H-1"), 4.01 (1H, dd, 13.3 and 3 Hz, H-5"$_{ax}$), 3.90 (m, 2H-6'), 3.82 (s, $CH_3O$), 3.65 (m, H-5'), 3.58 (dd, 13.3 and 1.5 Hz, H-5"$_{eq}$), 2.13 (s, 3H), 2.05 (s, 6H), 2.01 (s, 3H), 2.00 (s, 3H), 1.80 (s, 3H) – singlets of six acetoxy groups.

Paeoniflorin (II), yield 0.15%. White amorphous powder with the composition $C_{23}H_{28}O_{11}$, $[\alpha]_D^{25}$ -13.17° (c 3.23; ethanol), $\lambda_{max}^{EtOH}$ 230, 275, 282 (sh.) nm. $^1$H NMR spectrum ($D_2O$, 200 MHz), δ: 7.92 (2H, d, 8 Hz, H-2", 6"), 7.58 (1H, t, 8 Hz, H-4"), 7.43 (2H, t, 8 Hz, H-3", 5"), 5.48 (1H, s, –O–CH–O–), 4.58 (2H, s, $-CH_2-$Bz), 4.49 (1H, d, 6.5 Hz, H-1'), 3.76 (1H, d, 12 Hz, H-6'), 3.58 (1H, dd, 12 Hz, H-6'), 3.3-3.1 (m, 4H of glucose), 2.62 (1H, d, 7 Hz, H-5), 2.43 (1H, dd, 10.9 and 7 Hz, H-6$_{eq}$), 2.23 (1H, d, 12.8 Hz, H-3$_{eq}$), 1.92 (1H, d, 10.9 Hz, H-6$_{ax}$), 1.83 (1H, d, 12.8 Hz, H-3$_{ax}$), 1.40 (3H, s, $CH_3$). Mass spectrum, m/z (%): 178 (37%), 161 (3), 150 (21), 122 ($M^+$ of benzoic acid (86), 105 (100), 77 (97).

β-Sitosterol (III). White acicular crystals with the composition $C_{29}H_{50}O$ ($M^+$ 414, 100%), mp 137-138°C (methanol).

Benzoic Acid (IV). White crystals with the composition $C_7H_6O_2$, mp 120-121°C (aqueous alcohol), mass spectrum, m/z (%): 122 ($M^+$, 85), 105 (100), 77 (83).

Gallic Acid (V). White crystals with the composition $C_7H_6O_5$ ($M^+$ 170, 100%), mp 248-250°C (methanol), $\lambda_{max}^{EtOH}$ 216, 273 nm.

Ethyl Gallate (VI). White crystals with the composition $C_9H_{10}O_5$, mp 156-158°C (aqueous alcohol), $\lambda_{max}^{EtOH}$ 276 nm. Mass spectrum, m/z (%): 198 ($M^+$ 73), 170 (29), 153 (100). With a solution of $FeCl_3$ this substance gave an intense deep blue coloration, which is characteristic for phenolic compounds with three vicinal OH groups.

Enzymatic Hydrolysis. A solution of 5 mg of one of the glycosides (I and II) in 1 ml of water was treated with 1 mg of β-glucosidase (Serva) and the mixture was left overnight. Compound (II) was cleaved completely with the formation of glucose and an aglycon, while compound (I) did not undergo enzymatic hydrolysis.

Acid Hydrolysis. Compound (I) (10 mg) was heated with 2% HCl at 100°C for 5 min. Glucose and arabinose were found in the hydrolysate (PC). The aglycon was extracted with ether and, after evaporation, it was obtained in the form of a liquid with the composition $C_8H_8O_3$ ($M^+$ 152).

The acetylation of compound (I) was achieved with acetic anhydride in the presence of pyridine; the product was treated with ice water and the precipitate was recrystallized from ethanol.

## LITERATURE CITED


1. Plant Resources of the USSR. Flowering Plants, Their Chemical Composition, and Use. The Paeoniacea–Thymelaeaceae Families [in Russian], Nauka, Leningrad (1986), p. 7.
2. P. Tomczyk and S. Kohlmünzer, Herba Polonica, 5, No. 3, 175 (1979).
3. G. G. Zapesochnaya, Khim. Prir. Soedin., No. 6, 723 (1982).
4. G. G. Zapesochnaya, Khim. Prir. Soedin., No. 1, 21 (1979).
5. T. Kariyone, M. Takahashi, and M. Takaishi, J. Pharm. Soc. Jpn. (Yakugaku Zasshi), 76, 917 (1956); Chem. Abstr., 49, 7980f (1957).
6. T. Kariyone, M. Takahashi, and M. Takaishi, J. Pharm. Soc. Jpn., 76, 920 (1956); Chem. Abstr., 49, 7980f (1957).
7. S. Shibata and M. Nakahara, Chem. Pharm. Bull., 11, No. 3, 372 (1963).
8. M. Kaneda, Y. Iitaka, and S. Shibata, Tetrahedron, 28, No. 16, 4309 (1972).
9. N. Aimi, M. Inaba, M. Watanabe, and S. Shibata, Tetrahedron, 25, No. 9, 1825 (1969).