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PHENOLIC COMPOUNDS OF *Geranium sanguineum*

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The chemical composition of the phenolic compounds of the epigeal part of blood-red geranium has been studied. The structure of the main substance of the polyphenolic complex of the plant has been established — bis(hexahydroxydiphenoyl)trigalloylglucose. The high antiviral and antitumoral activity of the total phenolic compounds of the geranium has been shown.

In recent years, with the appearance of new methods of investigation, work in the field of the chemistry of phenolic compounds has received a new impulse. Simultaneously, great attention has been devoted to expanding the range of use of this group of natural compounds.

Preparations based on phenolic compounds can be used for reducing cholesterol levels [1] and for the prophylaxis and treatment of staphyloccal infections [2] and dysentery [3], and they possess antimutagenic [4, 5], antiherpetic [6], antiallergic [7], antihepatotoxic [8], and antitumoral [9, 10] actions. Researchers of various countries have devoted particular attention to the antiviral [11-14] activity of phenolic compounds, including the possibility of using them for the treatment and prevention of the human disease AIDS [15-19].

We have studied the composition of the phenolic compounds of *Geranium sanguineum* with the aim of creating from them drugs with an antiviral, interferon-inducing, and antitumoral action.

It was found that the amount of polyphenols in the leaves of the plant is 9-11%, in the stems 2. 5-3.5%, and in the roots 16-18%.

With the aid of two-dimensional PC in solvent systems 1 and 2 it was shown that the polyphenols of the epigeal part were represented by 13 compounds — flavonols, phenolic acids, and hydrolyzable tannins and their biogenetic precursors. For their study, we isolated the total phenolic compounds crom the epigeal part of the plant. Part of the material was subjected to fractionation on a column of hide powder. The column was washed successively with diethyl ether, water, pure acetone, and 60% aqueous acetone. From the ether fraction we isolated a white crystalline substance which, from its melting point, R_f values in various systems of solvents, and absence of a depression of a mixed melting point with an authentic sample was identified as gallic acid.

The complex of phenolic compounds isolated from the aqueous fraction consisted of a finely disperse powder and contained mainly three substances, with R_f 0. 78, 0.68, and 0.42 in solvent system 1. It was shown by qualitative reactions (ammonia vapor, Na_2CO_3 solution, UV light, etc.) that all these compounds were flavonoids. Chromatography of the preparation on a polyamide column using solvent systems 3 and 4 permitted the isolation of the flavonoid compounds in the individual state. On the basis of the results of a study of physicochemical properties and a comparison of with authentic samples, these substances were identified as kaempferol, quercetin, and rutin.

The total polyphenols isolated from the acetone fraction contained substances with R_f 0. 56 and 0.40 and traces of substances with R_f 0.33, 0.27, and 0.21. The last three compounds were identified on the basis of their chromatographic behavior in various solvent systems and by comparison with authentic samples as 2-galloylglucose, 2,3-digalloylglucose, and 3-galloylglucose. The complex of polyphenols isolated from the aqueous acetone fraction contained mainly two compounds, with R_f 0.40 and 0.37. It was chromatographed on a column of silica gel using systems 5 and 6. Fractions were obtained which

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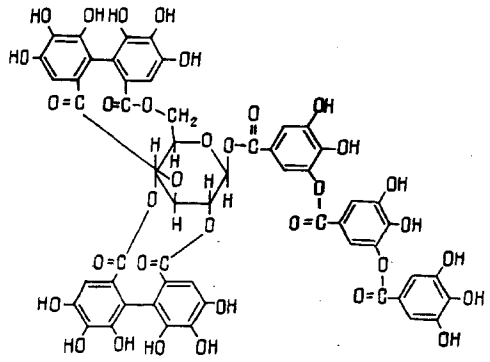
contained the substance with R_f 0.40 and a trace of the substance with R_f 0.37 and fractions containing the substance with R_f 0.37. The purification of the substances R_f 0.40 from contamination with the substance having R_f 0.37 was achieved by treating the fractions with activated carbon.

The substance with R_f 0. 40 was isolated by its precipitation from a Na_2SO_4-dried solution with a fivefold volume of petroleum ether. The compound isolated consisted of a light yellow finely disperse powder having mp 350°C (decomp.). UV spectrum, λ_{max}^{Me}, nm, (log ε) 217 (4.75), 257 (4.40). In the products of the complete acid hydrolysis of the substance we detected glucose and ellagic and gallic acids in a ratio of 1:2:3. The amount of ellagic acid was determined gravimetrically, that of gallic acid colorimetrically, and that of glucose with the aid of a micro method for determining sugars [20].

In the PMR spectra of the substance (acetone-d_6 there were the signals of glucose protons at (ppm) 6. 50 (1H), 5.55 (3H), 4.80 (2H), 5.55 (3H), 4.80 (2H), and 4.23 (1H), linked by spin-spin interactions, as followed from double-resonance experiments. Overlapping signals of the aromatic protons of gallic acid residues and of hexahydroxydiphenoyl groups were located in the 7.20-6.69 ppm region. In the spectrum of an acetyl derivative, only a slight change was observed in the chemical shifts of the glucose signals and an appreciable downfield shift of the signals of the aromatic protons. This showed that all the positions of the glucose in the molecule of the substance were substituted, and only the phenolic hydroxyls were acetylated.

The spectral and chemical results that we obtained were close to those of Japanese workers for geranin, isolated from *Geranium thunbergii*. The compound that we studied differed from geranin by the presence of three galloyl residues (geranin contains one). Therefore, by analogy, the following structure is proposed as the most probable for the substance under consideration.



Together with the Pacific Ocean Institute of Bioorganic Chemistry of the Russian Academy of Sciences, and the Vektor Scientific Production Combine of the Russian Ministry of Health, we have made an investigation of the preparation on systems modeling antiviral activity. It suppresses DNA polymerase activity with 100% specificity and has no effect on the activity of RNA polymerase. In a study of antiviral activity on a model system with reverse transcriptase the preparation completely inhibited the activity of the latter, which shows its promising nature as an agent possessing specific antiviral activity.

The antiviral activity of the preparation has been determined in the Scientific-Research Institute of Experimental Diagnosis and Chemotherapy, All-Russian Oncological Scientific Center, Russian Ministry of Health. The mean index of the inhibition of the incorporation of labeled thymidine into cell DNA (concentration $0.5 \cdot 10^{-4}$ M, time of exposure 24 h), was 83.8%.

EXPERIMENTAL

For column chromatography we used type L 40/100 silica gel and polyamide and hide powders. The qualitative compositions of the polyphenols were determined by the PC method.

Solvent systems: 1) *n*-butanol–acetic acid–water (40:12:28); 2) 15% acetic acid; 3) chloroform–methanol (9:1); 4) chloroform–methanol (8:2); 5) diethyl ether–ethyl acetate (1:1); 6) ethyl acetate. Revealing agents: 1) 1% alcoholic solution of $FeCl_3$; 2) mixture of 1% aqueous solutions of $FeCl_3$ and $K_3Fe(CN)_6$ (1:1); 3) ammonia vapor; and 4) 5% aqueous solution of Na_2CO_3.

The melting points of the compounds isolated were determined on a Boëtius instrument with a PHHK-9,5 visual apparatus (Germany). UV spectra were taken on a SF-26 spectrophotometer; PMR spectra were recorded on XL-200 instrument (Varian).

The air-dry comminuted epigeal part of the plant (300 g) was extracted with chloroform (3 × 2 liters at 50°C) to eliminate lipophilic substances. Then the raw material was dried and was extracted with 70% aqueous acetone (3 × 1.5 liters) at 40-45°C. The extracts were combined and were concentrated under reduced pressure in a current of nitrogen at 40-45°C. The aqueous residue was additionally treated with chloroform and then repeatedly (6 × 300 ml) with ethyl acetate. The combined ethyl acetate extracts were dried with anhydrous Na_2SO_4 and concentrated to small volume (300 ml), and the polyphenols were precipitated with a threefold volume of dry chloroform. The precipitate was filtered off, washed with dry chloroform, and dried in a vacuum-drying chamber at room temperature. The yield of the polyphenol preparation was 21.5 g (7.1% on the weight of the air-dry raw material).

The total polyphenols so obtained (10 g) were dissolved in 200 ml of hot (40°C) distilled water. Then the extract was cooled to room temperature and the total volume was made up with water to 800 ml. Hide powder (75.0 g) was covered with distilled water (900 ml) and the mixture was shaken in a shaking machine for 20 min, after which the hide powder was pressed out and was mixed with 800 ml of the solution of polyphenols that had been obtained and the mixture was shaken on the shaking machine for 45 min. After this, the hide powder with the substances absorbed on it was placed in five chromatographic columns with dimensions of 4.5 × 100 cm and was washed successively with diethyl ether, and distilled water until the reaction for phenolic compounds was negative. Elution was carried out with pure acetone and with 60% aqueous acetone. The course of the separation of the phenolic compounds was monitored by PC in system 1. The fraction eluted by ether contained a single substance, which, when a chromatogram was treated with revealing agent 1, gave a blue coloration, while with revealing agent 2 it gave a dark blue coloration. By evaporating the ether fraction (800 ml) to dryness and recrystallizating the residue from water, we obtained 0.05 g of a white crystalline substance with mp 239°C, R_f 0.72 and 0.56 in systems 1 and 2. It gave no depression of the melting point with an authentic sample of gallic acid.

The fraction eluted by water (1. 7 liter) was repeatedly (6 × 500 ml) treated with ethyl acetate. The ethyl acetate extract was dried with anhydrous Na_2SO_4 and was concentrated under vacuum at 45°C to small volume (300 ml) volume, and the phenolic compounds were precipitated by the addition of a fivefold volume of chloroform. The precipitate was filtered off and was dried in a vacuum-drying chamber at 35-40°C for 5 h. This gave 3.1 g of total polyphenols, a solution of 1.5 g of which in 15 ml of methanol was mixed with 10.0 g of polyamide powder and deposited on a chromatographic column (3.0 × 60.0 cm) of polyamide (50.0 g). The column was washed with systems 3 and 4. The fractions containing individual substances were combined and evaporated under vacuum to dryness, and the residues were recrystalized from aqueous ethanol (1:1). Three pale yellow microcrystalline substances were obtained that were identified from their melting points, mixed melting points, and UV spectra as kaempferol, quercetin, and rutin.

The aqueous acetone fraction, (2. 2 liters) obtained on elution of the polyphenols from the hide powder was concentrated under reduced pressure in a current of nitrogen at 40°C. The aqueous residue was treated with ethyl acetate, and the ethyl acetate extract (0.8 liter) was concentrated, dried with freshly calcined Na_2SO_4 (200 g), evaporated under vacuum to small volume, and mixed with a fivefold volume of petroleum ether. After filtration and drying of the precipitate that deposited, 1.27 g of a light cream amorphous powder was obtained.

A solution of this powder (1. 0 g) in 10 ml of acetone was mixed with 10 g of silica gel and the mixture was dried in a vacuum desiccator until the solvent had been completely eliminated. The silica gel with the absorbed substance was transferred to a column (2.5 × 50 cm, 50 g of silica gel) and the column was washed successively with systems 5 and 6. The course of separation was checked by PC in system 1. Fractions with a volume of 70 ml were collected. Fractions 7-12 contained a substance with R_f 0.40 with traces of a substance with R_f 0.37, and fractions 14-21 contained the substance with R_f 0.37. Fractions 7-12 were combined, concentrated, and rechromatographed on a silica gel column (2.5 × 40 cm, 40 g of silica gel). Then the eluate was concentrated to 80 ml and was treated with activated carbon (1.0 g) and filtered, and the substance was precipitated with a fivefold volume of petroleum ether. This precipitate was filtered off on a Schott No. 3 funnel and was dried in a vacuum desiccaator. The substance obtained (0.33 g) had mp >350°C (decomp.), λ_{max}^{Me} 217 nm (4.75), 257 nm (4.40).

For the performance of acid hydrolysis,). 15 g was dissolved in 30 ml of 5% H_2SO_4 and the solution was heated in a boiling bath in a flask fitted with a reflux condenser in a current of nitrogen for 1.5 h. The ellagic acid that then deposited was filtered off on a Schott No. 3 funnel, washed with hot distilled water, and dried in a vacuum desiccator over freshly calcined $CaCl_2$. Yield 0.066 g, which amounts to 91% of the calculated content of ellagic acid in $C_{55}H_{36}O_{34}$.

Gallic acid was determined photocolorimetrically. In the solution analyzed we found 0.051 g of gallic acid, which amounts to 93% of the calculated content of gallic acid in $C_{55}H_{36}O_{34}$.

Glucose was determined by the micro method for determining sugars. When 3 ml of the solution being analyzed was titrated with potassium permanganate, 0.740 mg of cuprous oxide was formed, which, according to a conversion table, corresponds to 0.185 mg of glucose. Hence, 30 ml of the solution contained 1.85 mg of glucose, which represents 88% of the calculated value for the amount of glucose in $C_{55}H_{36}O_{34}$.

The analysis of the compound isolated corresponded to the calculated figures.

REFERENCES

1. Japanese Patent No. 62-30711.
2. Japanese Patent No. 62-328330.
3. Japanese Patent No. 62-328327.
4. Japanese Patent No. 63-271820.
5. Japanese Patent No. 62-333210.
6. T. Nanabu, Phytochemistry, **24**, No. 10, 2245 (1985).
7. Japanese Patent No. 58-70774.
8. K. Chakraborti, Indian Drugs, **27**, No. 3, 161 (1989).
9. Japanese Patent No. 62-190166.
10. FRG Patent No. 380059657.
11. French Patent No. 8715370.
12. French Patent No. 8906282.
13. French Patent 8907654.
14. L. Van Hoof, Arch. Int. Physiol. Biochem., **95**, No. 5, 249 (1987).
15. Japanese Patent No. 62-161572.
16. FRG Patent 38001497.
17. Japanese Patent No. 62-321516.
18. Japanese Patent No. 62-321547.
19. Japanese Patent No. 62-311038.
20. A. I. Ermakov, Methods for the Biochemical Investigation of Plants [in Russian], VO Agropromizdat, Leningrad (1987), p. 430.
21. T. Okuda, T. Yoshida, and H. Nayeshiro, Chem. Pharm. Bull., **25**, No. 8, 1862 (1977).