# FLAVONOID COMPOUNDS OF Scutellaria przewalskii

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Scutellaria przewalskii Juz., family Labiatae is a perennial herbaceous plant growing in Kirgizia.

This paper gives the results of the isolation and chemical investigation of the flavonoid compounds of the epigeal part of Scutellaria przewalskii (studied for the first time) collected in 1963 during the flowering phase (Susamyr Valley).

The flavonoid compounds of Scutellaria attracted attention long ago but, in spite of numerous investigations, a comparatively small number of substances have been isolated and characterized. For example, scutellarin, baicalin, vogonin, chrysin, and their dihydro forms have recently been detected [1].

From the results of two-dimensional chromatography and qualitative reactions with zirconyl nitrate and ammonia, the flavonoid compounds of an ethanolic extract consist of derivatives of scutellarein (substances I, and V) and apigenin (substances II and III). Contrary to expectations, their total amount proved to be comparatively low.

Since the majority of the flavonoid glycosides of Scutellaria are glucuronides [1], we assumed that these compounds are present in the plant in the form of salts more soluble in water than in ethanol. Consequently, after ethanolic extraction the raw material was treated with water. Eight glycosides of a flavonoid nature were found in a considerable amount in the aqueous extracts. The aqueous solution was evaporated to small volume and diluted with 50% acetic acid. On there was a copious precipitate of light-yellow crystals of a mixture of flavonoids consisting of four glycoside derivatives of apigenin, luteolin, and scutellarein (fraction 1). The combined glycosides were highly soluble in a saturated aqueous solution of sodium bicarbonate and were reprecipitated from it on acidification. Consequently, it may be assumed that the carbohydrate substituent in them is glucuronic acid. Glycosides insoluble in aqueous sodium bicarbonate remained in the acetic acid mother liquor (fraction 2).

The glycosides of each fraction were first hydrolyzed and, together with apigenin, luteolin, and scutellarein, fraction 1 yielded substance A and fraction 2 substances B and C. According to qualitative reactions and chromatographic and spectral characteristics (Table 1), and also from the products of demethylation and alkaline cleavage, substance A was identified as dinatin (4'-methoxyscutellarein) [2],* substance B as hispidulin (6-methoxyscutellarein) [3], and substance C as pectolinarigenin (6,4'-dimethoxyscutellarein) [4].

The glycosides were separated by column chromatography on a polyamide sorbent on being eluted by 30% acetic acid (fraction 1) and 15% acetic acid (fraction 2), and eight monosides and three biosides were obtained.

The structures of the isolated compounds were studied by means of the products of acid, alkaline, and enzymatic hydrolysis, and UV spectroscopy with complex-forming and ionizing reagents (see Table 1). The carbohydrate substituents present in position 7 of the monosides were D-glucose or D-glucuronic acid, and in the biosides they consisted of glucose alone.

*Dinatin and hispidulin were identified by comparing their properties with those given in the literature [2, 3] and pectolinarigenin with a sample obtained by us [4] from Linaria officinalis L. common toadflax).

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TABLE 1. Spectral Characteristics of the Flavonoids of Scutellaria przewalskii

| Flavonoid | Ethanolic solut. | Ethanolic solution + sodium acetate | | Ethanolic solution + sodium ethanide | | Ethanolic solution + zirconyl nitrate | |
|---|---|---|---|---|---|---|---|
| | $\lambda_{max}$, nm | $\lambda_{max}$, nm | $\Delta\lambda$ | $\lambda_{max}$, nm | $\Delta\lambda$ | $\lambda_{max}$, nm | $\Delta\lambda$ |
| Apigenin | 336 | 380 | 44 | 400 | 64 | 390 | 54 |
| | 270 | 274 | 4 | 275 | 5 | 285 | 15 |
| Apigenin 7-glucoside | 335 | 335 | 0 | 400 | 65 | 390 | 55 |
| | 270 | 270 | 0 | 275 | 5 | 282 | 12 |
| Apigenin 7-glucuronide | 336 | 336 | 0 | 400 | 64 | 390 | 54 |
| | 270 | 270 | 0 | 276 | 6 | 285 | 15 |
| Luteolin | 350 | 380 | 30 | 410 | 60 | 400 | 50 |
| | 265 | 270 | 5 | 271 | 6 | 276 | 11 |
| | 255 | | | | | | |
| Luteolin 7-glucoside | 350 | 350 | 0 | 410 | 60 | 400 | 50 |
| | 267 | 270 | 3 | 270 | 3 | 275 | 8 |
| | 255 | | | | | | |
| Scutellarein | 335 | 370 | 35 | 370* | 35 | 375 | 40 |
| | 285 | 275 | −10 | 275 | −10 | 307 | 22 |
| Scutellarin | 335 | 333 | − 2 | 370* | 35 | 365 | 30 |
| | 282 | 286 | 4 | — | — | 305 | |
| Scutellarein 7-glucoside | 335 | 335 | 0 | 370* | 35 | 363 | 30 |
| | 280 | 285 | − 5 | — | — | 305 | |
| Scutellarein 7-glucobioside | 335 | 335 | 0 | 370* | 35 | 365 | 30 |
| | 280 | 285 | 5 | — | — | 303 | |
| Dinatin | 325 | 360 | 35 | 370** | 45 | 360 | 35 |
| | 275 | 270 | − 5 | 260 | −15 | 290 | 15 |
| Dinatin 7-glucoside | 325 | 325 | 0 | 365** | 40 | 360 | 35 |
| | 275 | 270 | − 5 | 260 | −15 | 290 | 15 |
| Dinatin 7-glucuronide | 325 | 325 | 0 | 365** | 40 | 360 | 35 |
| | 275 | 270 | − 5 | 260 | −15 | 290 | 15 |
| Dinatin 7-glucobioside | 325 | 325 | 0 | 365 | 40 | 360 | 35 |
| | 275 | 270 | − 5 | 260 | −15 | 290 | 15 |
| Pectolinarigenin | 330 | 365 | 35 | 370** | 40 | 355 | 25 |
| | 275 | 275 | 0 | 275 | 0 | 305 | |
| Hispidulin | 335 | 370 | 35 | 370* | 35 | 355 | 20 |
| | 285 | 275 | −10 | 275 | −10 | 305 | |
| Hispidulin 7-glucobioside | 335 | 335 | 0 | 370* | 35 | 355 | 20 |
| | 280 | 275 | − 5 | 275 | − 5 | 305 | |

*High-intensity absorption maximum.
† Low-intensity absorption maximum.

TABLE 2. Polarimetric Analysis of the Glucuronides

| Glucosides | $[\alpha]_D$, deg | $[M]_D$, deg | $K_{ph}$ | $[M]_D$, $K_\phi$ deg | Config. of bond | Form of sugar |
|---|---|---|---|---|---|---|
| Scutellarin | −102,0 | − 471 | 0,54 | − 254 | β | Pyranose |
| Phenyl D-glucuronide | − 91,0 | −228 | 1,00 | −228 | β | |
| Phenyl D-Glucuronide | +154,0 | +385 | 1,00 | +385 | α | |

The main glucuronide was scutellarin and, therefore, a polarimetric analysis was carried out on the basis of scutellarin which showed that the glucuronic acid in the glucosides is present in the pyranose form and has the $\beta$ configuration for the glycoside bond (Table 2).

Thus, from the epigeal part of Scutellaria przewalskii, in addition to the known scutellarin, we have isolated the following flavonoid compounds which are new for this genus: dinatin, hispidulin, pectolinarigenin, apigenin, luteolin; the 7-glucuronides of dinatin, apigenin, and luteolin; the 7-glucosides of scutellarein, dinatin, apigenin, and luteolin; and the 7-glucobiosides of scutellarein, dinatin, and hispidulin. Of the glycosides mentioned, all the biosides as well as the 7-glucuronide and the 7-glucoside of dinatin proved to be new.

## EXPERIMENTAL

To analyze the flavonoid compounds, the primary extracts were prepared in 70% ethanol and were chromatographed in the following systems: 1) 15% acetic acid, 2) butan-1-ol–acetic acid–water (4 : 1 : 2), 3) 60% acetic acid, and 4) 50% formic acid. The flavonoid aglycones were separated in system 5) benzene–

ethyl acetate–acetic acid (30 : 70 : 2), on paper impregnated with formamide (20% ethanolic solution of formaldehyde); the sugars in system 6) butan-1-ol – pyridine – water (6 : 4 : 2), and the aromatic acid in system 7) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4), and in system 5.

The spots of the flavonoids were developed with a 2% methanolic solution of a zirconyl salt and with $NH_3$ vapor. The spots of the scutellarein derivatives had a dark brown color in filtered UV light which changed little under the action of the reagents mentioned; the apigenin glycosides had a greenish-yellow color, and the luteolin glycosides an orange-yellow color.

Isolation of the Flavonoids. After treatment with 70% ethanol, 500 g of the comminuted raw material was extracted with hot water (3 × 2 liters). The combined aqueous extracts were evaporated to a viscous residue (200 ml) and diluted with two volumes of 50% acetic acid. On standing, the solution deposited a light-yellow crystalline precipitate (fraction 1), consisting of four substances with $R_f$ 0.08, 0.10, 0.12, and 0.18 (system 1).

The mother liquor was found to contain seven substances, with $R_f$ 0.08, 0.10, 0.16, 0.20, 0.30, 0.40, and 0.50 (fraction 2). The flavonoids of fraction 1 were separated on a column of polyamide with 30% acetic acid as eluent and those of fraction 2 with 15% acetic acid. The substances of fraction 1 were separated into four zones, each of which was cut out, after which the acid was eluted with water and the flavonoids with 70% ethanol. The substance of the second (from the bottom) zone separated out from concentrated solution in the crystalline form with mp 309-311°C, $[\alpha]_D^{20}$ – 102° (c 0.195, dimethylformamide), $R_f$ 0.12 (1), 0.31 (2), 0.29 (3). The three other substances were obtained in an amorphous state.

From fraction 2 five clear zones were treated as described above, giving the following individual compounds: a dinatin bioside with $R_f$ 0.50 (1) (first zone from the bottom), a scutellarein bioside with $R_f$ 0.30 (1) (second zone), and a hispidulin bioside with $R_f$ 0.40 (1) (third zone). The biosides were freed from impurities by rechromatography. The fourth zone contained monoglycosides of apigenin with $R_f$ 0.20 (1) and of luteolin with $R_f$ 0.10 (1), and the fifth contained mainly monoglycosides of scutellarein with $R_f$ 0.16 (1) and dinatin with $R_f$ 0.08 (1).

The separation of the monoglycosides was achieved by rechromatography on a polyamide sorbent under the conditions given. The glycosides were isolated in very small amounts in the amorphous state.

Acid Hydrolysis of the Glycosides. The total glucuronides and the individual glycosides of the first fraction were hydrolyzed by the method of Goldschmidt and Zerner [5]. The combined glycosides (0.5 g) were dissolved in 5 ml of conc. $H_2SO_4$ in the cold, and after 30 min the solution was carefully poured into 50 ml of cold water. The precipitate that deposited was separated off and subjected to chromatographic analysis and separation on polyamide with elution by 70% methanol. Apigenin with mp 347-349°C, $R_f$ 0.82, dinatin with mp 273-275°C, $R_f$ 0.78, luteolin with mp 329-330°C, $R_f$ 0.65, and scutellarein with mp 348-350°C (decomp.), $R_f$ 0.50 were isolated (system 5 for all the aglycones).

The aqueous part of the hydrolyzate was neutralized with barium bicarbonate, the precipitate of barium sulfate was filtered off, and the solution was evaporated to 1 ml. The concentrated solution was diluted with 10 ml of ethanol, and the new precipitate was separated off. The alcoholic filtrate was evaporated to 1 ml and analyzed for sugars. No neutral sugars were found. The substances precipitated by ethanol were dissolved in water (10 ml) and treated with KU-2 ion-exchange resin ($H^+$). The acid solution (pH 2) was evaporated to dryness and the residue was dissolved in 0.5 ml of ethanol. Chromatography on paper in comparison with an authentic sample in systems 3, 7, and 8 showed only the presence of glucuronic acid.

From the individual glycosides of this fraction under the same conditions of hydrolysis and working up we obtained D-glucuronic acid, scutellarein, dinatin, luteolin, and apigenin.

The glycosides of the second fraction were hydrolyzed with 10% $H_2SO_4$ in 50% ethanolic solution with heating in a water bath for 4-6 h. The hydrolyzate was then worked up as described above. The carbohydrate substituents of the glycosides proved to consist of D-glucose, and among the aglycones, in addition to scutellarein, dinatin, apigenin, and luteolin, we found and isolated hispidulin with mp 290-292°C, $R_f$ 0.58, and pectolinarigenin with mp 219-221°C, $R_f$ 0.90 (system 5).

Alkaline Cleavage of the Scutellarein Derivatives. Samples of scutellarein, dinatin, hispidulin, and pectolinarigenin (0.01 g each) were each dissolved in 5 ml of 20% aqueous KOH and the solutions were heated on a water bath in a current of nitrogen for 4 h. After neutralization the cleavage products were

extracted with ether and analyzed by paper chromatography (systems 5 and 8) in comparison with authentic p-hydroxybenzoic and anisic acids. The acids were also obtained preparatively by vacuum sublimation at 200°C. The purity of the acids was checked by chromatography. Scutellarein and hispidulin gave p-hydroxybenzoic acid, and dinatin and pectolinarigenin gave anisic acid. The identities of the acids were also confirmed by their UV spectra in methanolic and alkaline solutions [6].

## CONCLUSIONS

The epigeal part of Scutellaria przewalskii has been shown to contain apigenin, luteolin, scutellarein, dinatin, hispidulin, pectolinarigenin; the 7-glucosides and 7-glucuronides of apigenin, luteolin, scutellarein, and dinatin; and also the 7-glucobiosides of scutellarein, dinatin, and hispidulin; and these substances have been isolated. It has been established that the glucuronides of the flavonoids are present in the salt form.

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