FLAVONES OF THE ROOTS OF Scutellaria baicalensis

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UDC 547.972

One of the groups of active substances from the roots of Scutellaria baicalensis Georg. (Baikal skullcap) is formed by the flavonoids, of which baicalin and wogonin have been isolated and identified previously [1, 2].

The known flavonoids of the roots of this skullcap consist of a group of compounds in which the B ring is unsubstituted and there are vicinal substituents in the A ring [3]. Such structural features are responsible for the specific properties of these flavones and their high lability and, probably, also show that only two flavones have been isolated from the roots of this plant.

We have established that the roots of the Baikal skullcap contain a variety of other flavonoids. To obtain them in the individual states, we have developed a specific sequence of fractional separation.

The aglycone fraction was extracted with ethyl acetate and the glycosidic fraction with water. The glycosidic fraction contained baicalin and other glycosides. The substances of the aglycone fraction were analyzed by paper chromatography. In view of the fact that complete separation was not achieved in a single system, we used countercurrent chromatography [4]. The aglycone fraction was found to contain more than 20 substances of flavonoid nature (I-XXII). Taking the different polarities of the individual groups of aglycones into account, we first performed their fractionation and then isolated the individual substances. Six individual flavone aglycones (X, XI, XII, XIII, XIIIA, and XIV) were obtained and were investigated chemically.

According to PMR spectroscopy (Table 1), substances (X) and (XIV) contained substituents in positions 5, 6, and 7, and (XIII) in positions 5, 7, and 8. Substances (XIII) and (XIV) each contained one methoxy group. From the number of acetyl groups in the acetyl derivatives, it was shown that substance (X) contains three and substances (XIII) and (XIV)* each contain two hydroxy groups.

The positions of the other hydroxy groups were determined from the bathochromic shifts in the presence of ionizing and complex-forming reagents (Table 2) by the differential method [5]. The UV spectra of the compounds investigated in a neutral medium were characterized by an intense maximum in the 270-280-nm region and by a maximum of inflection of low intensity in the 315-355-nm region. By using this method it was possible to trace bathochromic shifts in the longwave region.

On ionization by sodium acetate, a 7-hydroxy group causes a bathochromic shift of the maximum of the first band by 50-80 nm. A 5-hydroxy group is detected from the bathochromy of zirconyl complexes.

The size of the shift in the compounds of the norwogonin series (XIII, XIIIA, and XII) is twice that of the flavones of the baicalein series (X, XI, and XIV) which enables a sharp distinction to be made between derivatives of baicalein (5,6,7-trihydroxyflavone) and norwogonin (5,7,8-trihydroxyflavone).

An analysis of the differential spectra of the boric acid complexes showed that they are formed with the participation of 6,7- and 7,8-dihydroxy groupings but do not arise in the case of 5,6-dihydroxy derivatives.

The alkaline reagent ionizes all the phenolic hydroxy groups, but in the case of norwogonin the substance rapidly decomposes. Apparently, a 5,7,8-trihydroxy grouping is more labile in an alkaline medium

* The NMR spectra of the initial substances (XIII) and (XIV) were interpreted by Prof. G. K. Nikonov.

Khar'kov Scientific-Research Institute of Pharmaceutical Chemistry. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 729-733, November-December, 1973. Original article submitted August 30, 1972.

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TABLE 1. Signals of the Protons of the Flavones Isolated

Acetates of	Protons at C (δ, ppm)						
	3	6	8	2', 6'	3', 4', 5'	$-OCH_3$	$-COCH_3$
Baicalein (X)	6.65s	–	7.49s	7.85m	7.55m	—	2.37s and 2.48s
Oroxylin (XIV)	6.63s	–	7.29s	7.85m	7.50m	3.89s	2.42s and 2.52s
Vogonin (XIII)	6.68s	6.83s	–	7.90m	7.53m	4.05s	2.42s and 2.46s
	6.57s	–	–	–	–	3.99s	–

TABLE 2. UV Spectra of Flavones of the Roots of Baikal Skullcap

Flavones	λ_{max} and $\Delta\lambda$, nm								
	in methanol	with sodium acetate		with alkali		with boric acid and sodium acetate		with zirconyl chloride	
	λ	λ	Δλ	λ	Δλ	λ	Δλ	λ	Δλ
Baicalein (X)	325 275	375 260	50 –	370 255	45 –	365 260	40 –	367 290	42 –
Oroxylin (XIV)	320 270	375	55	377	57	320		367	47
7-O-Methylbaicalein (XI)	340 275	340 275		410*		340 275		380	40
Norwogonin (XIIIA)	355 280	435	80	335	−20	410	55	475 310	120
Wogonin (XIII)	315 275 245	395 290	80	400 295	85	315 275		415 335 295	100
7-O-Methylnorwogonin (XII)	340 275	340 275		415	75	340 275		410	70

*Decomposes.

than the 5,6,7-trihydroxy grouping in baicalein. This is also confirmed by the change in the colors of the spots on chromatograms where baicalein with alkali acquires a greenish-brown color and norwogonin dark brown changing to blue. Flavone aglycones with free 8- or 6-hydroxy groups are easily detected on chromatograms from the blue color of the spots with a mixture of 1% aqueous solutions of ferric chloride and potassium ferrocyanide.

The natural monomethyl ethers of baicalein and norwogonin were demethylated with pyridinium chloride to the initial flavones, which served as a proof of their origin [6]. Norwogonin was obtained in the crystalline state under the same conditions from wogonin. Secondary derivatives were the acetates, which were used in NMR spectroscopy to determine the numbers of acetyl and methoxy groups (see Table 1).

The completeness of the replacement of the hydroxy groups and, consequently, the individuality of the acetyl derivatives has to a large extent been checked hitherto only by means of qualitative reactions or by IR spectroscopy. We have also used the chromatographic analysis of the acetates obtained.

The six flavonoids that we isolated have been characterized as 5,6,7-trihydroxyflavone, or baicalein (X), 6-O-methylbaicalein, or oroxylin (XIV), 7-O-methylbaicalein (XI), 5,7,8-trihydroxyflavone or norwogonin (XIIIA), 8-O-methylnorwogonin, or wogonin (XIII), and 7-O-methylnorwogonin (XII).

Of these compounds, we are the first to have found oroxylin in a skullcap and the first to have isolated the 7-O-methylderivatives of baicalein and norwogonin from plants.

The amounts of the individual flavone aglycones were determined spectrophotometrically after their

chromatographic separation [3]. The amount of baicalein was 2%, of oroxylin 2%, of wogonin about 1%, and of 7-methoxybaicalein about 0.5%.

EXPERIMENTAL

Qualitative Analysis of the Flavonoid Aglycones. A 10-g sample of the roots of Scutellaria baicalensis comminuted by roll-crushing to flakes with a size of 1-2 mm was exhaustively treated with ethyl acetate. The extracts (100 ml) were evaporated to 10 ml, and the flavonoid aglycones were analyzed by chromatography on paper impregnated with formamide by the method of paper countercurrent chromatography. For this purpose, a strip of paper (20 × 60 cm) was divided into two equal parts by a crease along its length and, at a distance of 2 cm from the line of the crease the extract was deposited on a starting line. Chromatography was first performed in the cyclohexane–benzene (7:3) system (1), whereupon the feebly polar compounds (XII-XV) were separated and the more highly polar compounds remained at the start. Then the part of the chromatogram with the separated substances was cut off and the remaining substances were chromatographed in the benzene–ethyl acetate–acetic acid (50:50:1) system (2). The substances (XII-XV) separated in system 1 had R_f values of 0.15, 0.30, 0.50, and 0.65, respectively, and the compounds (I-XI) separated in system 2 had R_f values of 0.15, 0.20, 0.28, 0.35, 0.50, 0.55, 0.65, 0.70, 0.75, 0.82, and 0.90.

Separation of the Flavonoid Aglycones. The roots of Scutellaria baicalensis (500 g) were treated with ethyl acetate (3 × 3 liters) as described above. The ethyl acetate extract was evaporated to dryness and the residue was dissolved in 100 ml of aqueous acetone (8:2). Taking the polarity of the aglycones into account, they were separated between aqueous acetone and petroleum ether. Substances (XII-XV) (fraction 1) passed into the first phase and substances (I-XI) (fraction 2) remained in the aqueous acetone. The substances of fraction 1 were chromatographed on Kapron impregnated with dimethylformamide in system 1. A series of subfractions was isolated of which two contained the individual substances (XIV) and (XIII). The eluates of the last fractions contained a mixture of substances (XII) and (XIII). These substances were separated by preparative chromatography in a thin layer of silica gel [chloroform–acetic acid (99:1) system].

Substance (XII), R_f 0.15 (1) and 0.89 (2). The color of the spot before treatment with chromogenic agents (in filtered UV light) was dark, and it changed little with 2% methanolic zirconyl chloride (Z) while with 10% methanolic alkali (K) it became brown and with a mixture of equal volumes of 1% aqueous solutions of ferric chloride and potassium ferrocyanide (FC) it became blue.

Substance (XIII), R_f 0.30 (1), 0.95 (2). Before treatment with chromogenic agents, the spot was dark, with reagent Z it changed little, with K it became brown, and with FC it gave no color, mp 201-203°C.

The substances of fraction 2 were chromatographed on a column (50 × 300 mm) of Kapron [eluent: acetone–water (8:2)], giving the subfractions 2A (I, IV, V, and VIII), 2B (II, III, VI, and IX), and 2C (X-XIII and XIV). Fraction 2-C was reseparated on a column (30 × 200 mm) of Kapron impregnated with dimethylformamide in system 1. The first portions contained substance (X) and the subsequent portions contained the pairs of substances (X) and (XI), (XII) and (XIII), and (XIII) and (XIV). The mixtures of substances (X) and (XI) were separated by preparative chromatography on paper in system 2.

Substance (X), mp 260-262°C, R_f 0.05 (1) and 0.82 (2). The color of the spot before treatment with chromogenic agent was dark, changing little with reagent Z, yellow-brown with K, and blue with FC.

Substance (XI), R_f 0.05 (1), 0.90 (2). The color of the spot before treatment with chromogenic agent was dark, changing little with reagent Z, yellow-brown with K, and not changing with FC. The UV spectra of the aglycones isolated are given in Table 2.

Acetates of Substances (X, XIII, and XIV). Solutions of 0.100 g of each of the substances mentioned in 2 ml of acetic anhydride were each treated with two drops of concentrated sulfuric acid and after 5 min the reaction mixtures were poured into tenfold volumes of distilled water cooled to 5-7°C. The precipitates that deposited were crystallized from aqueous acetone.

The individuality of the products and the completeness of the acetylation were checked by chromatography on paper impregnated with formamide in cyclohexane. The acetate of substance (X) had mp 194-196°C, R_f 0.10; that of substance (XIII) mp 118-120°C, R_f 0.30; and that of substance (XIV) mp 141-143°C, R_f 0.40. The color of the spots of the acetates on the chromatograms in UV light before treatment with chromogenic reagents was light yellow.

Demethylation of Substance (XIII). Substance (XIII) (0.200 g) was dissolved in a melt of pyridinium chloride (2 g) and the mixture was heated at 180-200°C for 50 min. After cooling, a tenfold volume of water was added to the reaction mixture. The precipitate that deposited was crystallized from methanol. The melting point of substance (XIIIA) was 254-256°C, R_f 0.05 (1) and 0.80 (2), the color of the spot before treatment with chromogenic agent was dark, with reagent Z it became dark brown and red-brown, changing to pale blue with K and deep blue with FC. The other substances were demethylated similarly. Compounds (XI) and (XIV) yielded substance (X), and (XII) yielded substance (XIIIA).

SUMMARY

1. A qualitative analysis of the flavonoid aglycones in the roots of Scutellaria baicalensis Georg. has been performed, and more than 20 substances have been detected in them.

2. Six individual flavonoid aglycones have been obtained, and on the basis of chemical and spectral characteristics they have been identified as baicalein, 7-O-methylbaicalein, oroxylin, norwogonin, 7-O-methylnorwogonin, and wogonin.

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