CHRYSIN AND ITS DERIVATIVES IN PLANTS OF THE GENUS *Scutellaria*

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Chrysin (5,7-dihydroxyflavone) — one of the simplest flavonoids — is found comparatively rarely in plants, and therefore the detection and identification of this compound and its derivatives is important in chemotaxonomic investigations [1].

In a study of the flavonoids of the genus *Scutellaria* L. (skullcap) a chrysin derivative was found as early as 1889 [2] and was characterized as 8-methoxychrysin or wogonin [3]. A second compound from the roots of *S. baicalensis* Georgi (Baikal skullcap) was 6-hydroxychrysin or baicalein [4]. Chrysin itself has been isolated from the epigeal part of *S. galericulata* L., but not until 50 years later [5]. Later still, in a study of the flavonoids of *S. epilobifolia* A. Hamilt., (a Canadian analog of *S. galericulata*) 2'-methoxychrysin was found in addition to chrysin itself [6].

On investigating the flavonoids of various species of skullcap, in addition to baicalein, oroxylin, norwogonin, wogonin, dihydronorwogonin, and their glucuronides, we observed the presence of chrysin and its 7-glucuronide [7, 8-10]. However, on comparing the properties of the chrysin in various species of skullcap substantial differences were found which induced us to perform a detailed study of them.

For the comparative study, the chrysin-like compounds (I) and (II) were isolated from the epigeal part of *S. scordiifolia* L. and (III) and (IV) from the roots of *S. granulosa* Juz. The physicochemical properties of (I)-(IV) are given in Table 1.

According to preliminary analysis, substances (I) and (II) were described as chrysin and its 7-glucuronide [8]. To confirm the structure of (I) we recorded and interpreted its PMR spectrum in $(CD_3)_2SO$ (Fig. 1a). It follows from the spectrum that (I) has free hydroxy groups at C-5 (s, 12.83 ppm, 1 H) and at C-7 (s, 10.90 ppm, 1 H), protons at C-3 (s, 6.93 ppm, 1 H), at C-8 (d, 6.52 ppm, J = 2.5 Hz, 1 H), at C-6 (d, 6.22 ppm, J = 2.0 Hz, 1 H), at C-2',6' (weakly resolved multiplet, 8.30 ppm, 2 H), and at C-3',4',5' (weakly resolved multiplet, 7.58 ppm, 3 H). In (II) (TMS ether) (Fig. 1b), in addition to information similar to that obtained for (I) the following signals were observed: the proton attached to the anomeric carbon atom (weakly resolved doublet at 5.0 ppm, J = 7 Hz, 1 H) and the protons of the remainder of a carbohydrate substituent (m, 3.3-3.4 ppm, 4 H).

The PMR spectrum of (III) [in $(CD_3)_2SO$] (Fig. 1c) showed the signals of the protons of hydroxy groups at C-5 (s, 12.84 ppm, 1 H) and at C-7 (s, 10.85 ppm, 1 H), and of the following aromatic protons: at C-6 (d, 6.20 ppm, J = 2.5 Hz, 1 H), at C-8 (d, 6.46 ppm, J = 2.5 Hz, 1 H), and at C-3 (s, 6.84 ppm, 1 H). A methoxy group was identified from a three-proton singlet at 3.94 ppm.

In view of the fact that in rings A and C of the flavonoid (III) all the positions for the substituents have been determined it may be assumed that the methoxy group is present in ring B. This was confirmed by the observation in the PMR spectrum of the signals of four protons additional to those observed previously.

Protons were found in ring B at C-4' (m, 7.60 ppm, $J_1 = J_2 = 8$ Hz, $J_3 = 2$ Hz, 1 H), at C-3' (t, 7.23 ppm, $J_1 = 8$ Hz, $J_2 = 2$ Hz, 1 H), at C-5' (m, 7.14 ppm, $J_1 = J_2 = 8$ Hz, $J_3 = 2$ Hz, 1 H), and at C-6' (quartet, 7.88 ppm, $J_1 = 8$ Hz, $J_2 = 2$ Hz, 1 H). The signals of four

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TABLE 1. Comparative Characteristics of the Flavonoids from Plants of the Genus Scutellaria*

Index	Aglycone and its glycoside			
	I	II	III	IV
Melting point	288–290°	228–230°	288–290°	200–202°
$[\alpha]_D^{20°}$	—	—80,0°	—	—75,0°
R_f x 100 in following systems 1	58–60	—	62–64	—
2	92–95	—	92–95	—
3	—	48–50	—	44–46
λ_{max} in methanol, nm	270, 310 sh.	270. 305 sh.	267, 330 sh.	270–330 sh.
with sodium acetate	375	—	385	—
with alkali	380	380	380	380
with zirconyl chloride	290	290	290	290
	340	330	—	—
	390	385	390	390

*From the roots of the oriental skullcap V. I. Glyzin et al. [11] isolated chrysin, with mp 250-255°C, and chrysin 7-glucuronide, with mp 194-195°C and $[\alpha]_D$ −84.5° (c 0.5; formamide); a synthetic sample of chrysin 7-glucuronide had mp 224-226°C, $[\alpha]_D$ −107° [12], and chrysin 7-glucuronide from *S. galericulata* [5] had mp 225-226°C and $[\alpha]_D$ −112° (c 1; dimethylformamide).

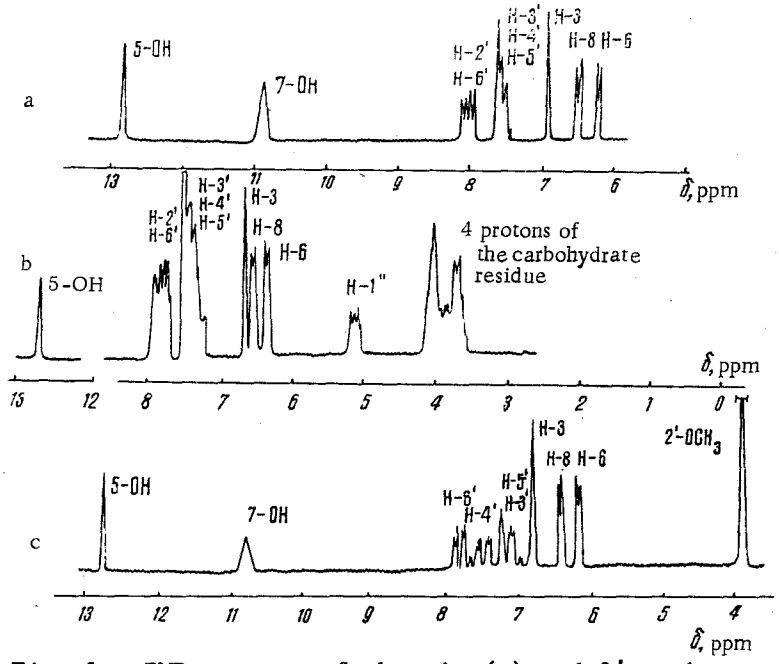

Fig. 1. PMR spectra of chrysin (a) and 2'-methoxychrysin (c) in DMSO and of the TMS ether of chrysin 7-glucuronide (b) in CCl_4.

adjacent protons of the aromatic ring are split as the result of ortho and meta interaction and have ortho and meta constants [13].

Thus, PMR spectroscopy has confirmed for (I) the structure of 5,7-dihydroxyflavone and for (III) the structure of 5,7-dihydroxy-2'-methoxyflavone.

The demethylation of (III) with pyridinium chloride [14] led to 2'-hydroxychrysin (V) which, in contrast to (I)-(IV) gave an intense blue coloration with Barton's reagent [15], revealing a feebly conjugated phenolic group. On chromatograms, (V) gives a spot that is yellow in filtered UV light (360 nm) in contrast to the dark brown for (I-IV) and it has R_f 0.05-0.07 (system 1) and 0.80-0.82 (system 2). In the UV spectrum (methanol)(V) has maxima

at 270 and 240 (sh.) nm; with sodium acetate 385 nm, with sodium acetate and boric acid no shift is observed; with alkali 405 nm, and with zirconyl chloride 290 and 390 nm.

The alkaline cleavage of (V) yielded salicylic acid and phloroglucinol (VI), while (I) gave (VI) and benzoic acid and (III-VI) each gave o-methoxybenzoic acid.

Contrary to a statement by Watkin [6], (V) differs distinctly from (I) and (III) by its chemical, chromatographic, and spectral properties, which gives grounds for doubting the reliability of the isolation of 2'-methoxychrysin from *S. epilobifolia*. It may be assumed that (III-V) are more characteristic for the species of the section Lupulinaria A. Hamilt. than for species of the section Galericularia A. Hamilt., to which *S. epilobifolia* belongs.

The glucuronides (II) and (IV) were cleaved by acids and by β-glucuronidase to glucuronic acid and the aglycones (I) and (III), respectively. Free carboxy groups in the carbohydrate moieties of glycosides (II) and (IV) are shown by bands in the IR spectrum in the 1725-1730 cm^{-1} region which disappeared when the glycosides were converted into the salt form. The PMR spectrum of the carbohydrate moiety of the glycoside (IV) is similar to that described for (II) (see Fig. 1b).

Glycosides (II) and (IV) were found to have double melting points. The first melting was observed in the ranges given in Table 1. At 240-250°C, crystallization from the melt took place, and the second melting was observed at 270-280°C. A double melting point is known for some flavonoid glycosides [16] and we attempted to determine its cause for the glycosides (II) and (IV). Samples of the glycosides were melted and, after crystallization from the melt, the products formed were separated and identified. It was found that the thermolysis of (II) forms the aglycone (I), and (IV) forms (III).

EXPERIMENTAL

The UV spectra of the flavonoids were taken in methanol on an SF-16 spectrophotometer by the differentiation method [9, 17].

The IR spectra (KBr) were recorded on a UR-10 spectrometer and the PMR spectra on a Hitachi–Perkin–Elmer R-20A instrument with a working frequency of 60 MHz. The solvents used were carbon tetrachloride and deuterated dimethyl sulfoxide. Trimethylsilane was used as internal standard.

The specific optical rotation was measured for 0.5% solutions in dimethylformamide on an SPU-E-M spectropolarimeter.

The aglycones were separated chromatographically on paper (type S ["medium"]) impregnated with formamide in the following systems: 1) benzene–hexane–acetic acid (70:30:1) and 2) benzene–ethyl acetate–acetic acid (50:50:1); and that of the glycosides (the paper was not impregnated) in system 3), acetic acid–water (15:85), or on columns of polyamide sorbent (polycaprolactam).

Isolation of the Glycosides (II) and (IV). Glycoside (II) was extracted from the comminuted air-dry epigeal part of *Scutellaria scordiifolia* with hot water (85-90°C, 2 × 6 liters, from 0.5 kg of plant material). The aqueous extract was evaporated to 1.5 liters and purified with ethyl acetate–ethanol (9:1) (two extractions of 1 liter each). The glycosides from the acidified purified aqueous solution (350 ml of 18% HCl) were extracted with ethyl acetate–ethanol (9:1) (3 × 1 liter). The ethyl acetate–ethanol solution was washed with water saturated with the same solvent (2 × 0.5 liter) and was chromatographed on a column of polyamide sorbent (5 × 50 cm, 200 g of powder with a particle size of 0.1-0.5 mm). The eluent was ethyl acetate, and 100-ml fractions were collected. Fractions 5-10 contained the glycoside (II). The eluates were evaporated to dryness and the glycoside (II) was crystallized from ethyl acetate. Yield 2.0-2.2% of the weight of the dry plant material.

Glycoside (IV) was isolated similarly from the dry comminuted roots of *S. granulosa*. Yield 1.6-1.8%.

Acid Hydrolysis of the Glycosides. A suspension of 1 g of glycoside (II) in 20 ml of a 10% solution of hydrochloric acid in 50% acetic acid was boiled under reflux on the water bath for 4 h. The cooled reaction mixture was diluted with an equal volume of water and the precipitate that deposited was filtered off. The yield of the aglycone (I) was 0.57 g.

The hydrolysis of glycoside (IV) was performed similarly, giving the aglycone (III) with a yield of 60% of the initial sample.

Enzymatic Hydrolysis of Glycosides (II) and (IV). A solution of 0.100 g of glycoside (II) in 5 ml of phosphate buffer (pH 7.0) was treated with 0.02 g of β-glucuronidase in 5 ml of buffer solution. Hydrolysis was performed in a thermostat at 36 ± 2°C for 16 h. The reaction mixture was diluted with an equal volume of ethanol and heated on the water bath to the boil (15 min). The amorphous precipitate that formed was separated off, and the filtrate was evaporated to an aqueous solution (7 ml). On standing, this deposited a crystalline precipitate (0.051 g) of the aglycone (I). The aqueous solution was evaporated to dryness and the residue was acidified with 0.5 ml of acetic acid and dissolved in 2 ml of ethanol. The precipitate of inorganic salts was separated off and the filtrate was used for the chromatographic analysis of the carbohydrate moiety of the glycoside. Paper chromatography [ethyl acetate—acetic acid—formic acid—water (8:3:1:4); chromogenic agent the aniline hydrogen phthalate reagent] showed two spots — with R_f 0.15, at the level of glucuronic acid (VII), and with R_f 0.45, at the level of γ-glucuronolactone (VIII). The hydrolysis of (IV) was performed similarly and the aglycone (III) was isolated while, in addition (VII), and (VIII) were detected.

Thermolysis of the Glycosides (II) and (IV). On a clock glass on the heating stage of a Kofler block, 0.050 g of glycoside (II) was heated at the rate of 4 deg/min (230°C) and heating was continued until complete recrystallization took place (245-250°C). At this stage, heating was stopped and the cooled product was dissolved in 20 ml of methanol. The methanolic solution was diluted with an equal volume of water and filtered through a layer of polyamide (column 2 × 30 cm, 50 g of sorbent). The unchanged part of the glycoside (II) and other insoluble decomposition products were eluted with 50% methanol (100 ml), and the aglycone was desorbed with methanol (100 ml). The methanolic eluate was evaporated to dryness and the aglycone was crystallized from 50% ethanol. Yield 0.022 g of the aglycone (I). The thermolysis of the glycoside (IV) was performed similarly and the aglycone (III) was isolated with a yield of about 50% of the initial sample.

Demethylation of the Aglycone (III). A mixture of 0.05 g of the aglycone (III) and 2 g of crystalline pyridinium chloride was heated in a round-bottomed flask (20 ml) with a reflux condenser in an oil bath at 180°C for 1 h. Then the mixture was cooled and dissolved in 20 ml of water. The precipitate that deposited on standing was separated off and washed on the filter with distilled water until pyridine had been completely eliminated. The yield of substance (V) was 0.03 g.

Alkaline Cleavage of the Aglycones (I), (III), and (V). In a porcelain crucible, 0.05 g of substance (I) was mixed with a melt of anhydrous caustic potash (2 g, fusion temperature 200°C). Then the mixture was cooled and dissolved in 10 ml of water. The solution was acidified with hydrochloric acid to pH 7.5 and the substances of the first fraction (phenols) were extracted with ethyl ether (3 × 20 ml). The ethereal solution was washed with ether-saturated water and evaporated to dryness.

The substances of the second fraction (aromatic acids) were extracted from the aqueous solution acidified to pH 5.0. The ethereal extract was washed with ether-saturated water, dried with anhydrous sodium sulfate, and evaporated to dryness at 35°C. The dry residue was purified by sublimation in a round-bottomed flask with a finger-type condenser in an oil bath at 100-120°C. A white crystalline substance with mp 122-124°C was isolated, and it was identified by qualitative reactions and chromatographic and spectral properties as benzoic acid. Phloroglucinol — identified by paper chromatography, by comparison with an authentic sample of phloroglucinol, and by qualitative reactions with ferric chloride, with vanillin in hydrochloric acid, etc. — was found in the residue from the first fraction.

The cleavage of substances (III) and (V) was performed similarly. From (III) we isolated and identified phloroglucinol and o-methoxybenzoic acid (mp 98-100°C, by sublimation), and from (V) phloroglucinol and salicylic acid (mp 158-160°C, by sublimation).

SUMMARY

1. A glycoside (II) identified by chrysin 7-β-glucopyranosiduronic acid has been isolated from the epigeal part of *Scutellaria scordiifolia* in a yield of 2.0-2.2%.

2. A glycoside (IV), characterized as 2'-methoxychrysin 7-β-D-glucopyranosiduronic acid has been isolated from the roots of *S. granulosa*.

3. This is the first time that chrysin and 2'-methoxychrysin and their 7-glucuronides have been isolated from the *Scutellaria* species mentioned.

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PROANTHOCYANIDIN DIMERS FROM *Spiraea hypericifolia*

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Continuing a study of the flavans of individual parts of *Spiraea hypericifolia* L., we have detected the presence in the bark and roots of this plant of three dimeric flavans and have established their structures as (—)-epicatechin-(+)-catechin (flavan I) and (—)-epicatechin-(—)-epicatechin (flavan II).

Like all flavans, the substances investigated give a red coloration with vanillin in concentrated HCl [1] and an insoluble phlobaphene on being heated with dilute mineral acids [2]. The alkaline cleavage of each of the compounds formed phloroglucinol and protocatechuic acid.

Heating the substances with 2 N HCl in methanol led to the formation of cyanidin, which was identified by paper chromatography in the solvent system hydrochloric acid—acetic acid—water (5:1:5) and by UV spectroscopy (λ_{max} 535 nm in ethanol). The substances differed in the products of their acid cleavage under mild conditions (0.05 N HCl): the flavan (I) formed (+)-catechin and traces of (—)-epicatechin and flavin (II) formed (—)-epicatechin.

Cleavage under mild conditions in an acid medium gave the catechin from the "bottom" part of the molecule in view of the fact that the conjugated orientation of the hydroxy

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