FLAVONOIDS OF THE RHIZOMES OF *Rhodiola rosea*.

I. TRICIN GLUCOSIDES

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Gallic acid and the flavonoid tricin (4',5,7-trihydroxy-3',5'-dimethoxyflavone) and its 7- and 5-O glucosides have been isolated for the first time from the rhizomes of *Rhodiola rosea* L. Identification was made on the basis of UV, IR, PMR, and mass spectra of the initial substances and the products of their acid hydrolysis and acetylation. The physicochemical constants, not previously given in the literature, of the 5-O-β-D-glucopyranoside have been determined.

Extracts of roseroot sedum (*Rhodiola rosea* L., family *Crassulaceae*) are widely used in folk and scientific medicine as a stimulant and adaptogenic agent [1], but the chemical composition of the plant has been studied inadequately. Phenolic compounds have been isolated from the rhizomes: tyrosol (p-hydroxyphenylethanol) and its glucoside, which was called rhodioloside [2, 3], and was then identified as the salidroside isolated previously from willow [4]. From the epigeal part of roseroot sedum have been isolated oxalic and succinic acids and the flavonoids kaempferol and astragalin; fumaric, gallic, tartaric, citric, malic, and lactic acids have also been detected [5].

We have investigated the components of an ethanolic extract of the rhizomes of roseroot sedum, and from it, by the successive use of several sorbents, three flavonoid compounds (I-III) and gallic acid (IV) have been isolated.

When the flavonoids were subjected to acid hydrolysis, compound (I) did not change, while (II) and (III) gave identical products [the aglycone (I) and glucose], (III) being hydrolyzed almost instantaneously and (II) in the course of 1.5 h. Compound (I) had the skeleton of a 3',4',5,5',7-substituted flavone with two methoxy groups (PMR spectrum) and three hydroxy groups (M^+ 330), of which one was located in position 5 (singlet at 13.0 ppm). In each of the PMR spectra [of compounds (I), (II), and (III) and the acetate of (III)], the two methoxy groups give a single singlet with an intensity of 6 H, and two other protons (H-2',6') likewise resonate in the form of a singlet, which indicates the symmetrical substitution of ring B, i.e., the structure 4',5,7-trihydroxy-3',5'-dimethoxyflavone (tricin) for compound (I). The presence of the methoxyls in the lateral ring was also shown by the fragmentary ions B (m/z 178) and A + H (m/z 153) in the mass spectrum of (I). The stability and the increase in the intensity of the long-wave maximum in the UV spectrum in the presence of sodium methanolate confirmed the presence of a free 4'-OH group, and a bathochromic shift of the short-wave maximum in the presence of sodium acetate indicated the presence of a free 7-OH group.

The PMR spectra of (II) and (III) contain a signal in the form of doublet with J = 7 Hz, which is characteristic for the anomeric proton of a β-D-glucopyranoside in the chair-like conformation $^4C_1$. A comparison of the UV spectra of compounds (I) and (II) made it possible to identify (II) as tricin 7-O-β-D-glucopyranoside [6].

The PMR spectrum of compound (III) in DMSO differed from that of (II) by the fact that it contained no characteristic signal of a 5-OH group and permitted the assumption of 5-glycosylation in (III). In the IR spectrum of (III), the band of a carbonyl group is located at 1635 cm^{-1}, while in (I) it is at 1655 cm^{-1}, which is due to a disturbance of the usual chelation of the 5-OH group. Furthermore, compound (III) had the bright blue fluorescence that permits 5-substituted flavones to be readily distinguished [7]. In many cases, the literature gives information on the "dual" melting points of 5-glycosides, but monitoring the

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heating process with the aid of TLC showed that the melting points of the initial glycosides are the first, after which the glycosides decompose with the formation of aglycones which then give the second, higher, melting points [8]. Compound (III) behaved similarly: it melted in the range of 173-177°C, the melt underwent crystallization at 185°C, and the aglycone melted at 264-269°C.

We have mentioned above the ready hydrolyzability of compound (III) in an acid medium. We also observed the decomposition of tricin 5-glucoside in the course of recording its UV spectrum in the presence of $AlCl_3$. With the usual method of recording, the spectrum obtained was practically that of the aglycone (with a bathochromic shift of the long-wave band), but when the long-wave part of the spectrum was recorded immediately after the addition of $AlCl_3$ we observed no shift of the band at 348 nm.

The comparatively large value of the specific rotation of glucoside (III) is more characteristic for β-D-glucofuranosides, but the PMR spectrum of its acetate in benzene-d_6 permitted all the signals of the carbohydrate protons to be identified, and their SSCCs left no doubt of the $^4C_1$ conformation of the β-D-glucopyranose residue. An additional proof of the tricin structure of the aglycone was the resonance of the methoxyls in the spectrum of the acetate of (III) (δ 3.92 in $CDCl_3$ and 3.51 in C_6D_6), which permits them to be assigned to positions 3', 5' [9]. Thus, compound (III) has the structure of tricin 5-O-β-D-glucopyranoside. It must be mentioned that both the glycosides isolated were hydrolyzed by β-glucosidase but in this case, unlike acid hydrolysis, the hydrolysis of the 7-glucoside took place faster than that of the 5-glucoside.

We are the first to have isolated tricin and its glucosides from roseroot sedum and there have been no reports on their presence in other representatives of the family *Crassulaceae*.

Its lability and ease of resinification and, on the other hand, its capacity for being strongly adsorbed on cellulose and other sorbents have prevented authors from isolating tricin 5-glucoside in appreciable amounts [7, 10]. Consequently no constants are given in the literature with the exception of the UV spectrum [10], which was later corrected [7], the authors explaining that the positive shift with $AlCl_3$ (erroneously given in the first paper [10]) was probably caused by the presence of free tricin in the sample of the 5-glucoside. In our opinion, here the decomposition of the sample during the recording of the spectrum that has been mentioned above possibly took place.

In publications by J. B. Harborne [10], it has been reported that flavonoid 5-glycosides are interesting taxonomic markers of higher plants, because of their relative rarity and ease of recognition. In not one of the plants and families studied were 5-glycosides detected in the roots, and their presence in the flowers and their capacity for fluorescing is connected with possible attractant properties [7].

EXPERIMENTAL

The spectral characteristics were obtained on the instruments Varian HA-100D at 100 MHz with tetramethylsilane as internal standard (PMR); Varian CH-8 at 70 eV (mass spectra); Hitachi EPS-3T (UV); and UR-20, paraffin oil (IR). Melting points were determined on Kofler block. Elementary analyses were performed on a Hewlett-Packard 185 B automatic CHN analyser. Angles of rotation were obtained on a Polamat A polarimeter at 546 nm and were recalculated by means of the formula $[\alpha]_D = [\alpha]_{546}/1.17543$. Chromatographic monitoring was carried out by GLC (Silufol) in the systems chloroform—methanol (6:1 and 9:1) and chloroform—methanol—water (26:14:3) and by PC (identification of sugars) in the systems butanol—acetic acid—water (4:1:2), butanol—pyridine—water (6:4:3), and water-saturated phenol.

Isolation. Rhizomes of *Rodiola rosea* L. were collected on September 28, 1978, at a height of 1800 m (Narymskii Range, East-Kazakh Province), and they were dried in comminuted form at 50-60°C. The dried rhizomes (885 g) were extracted with ethanol, after which vacuum evaporation yielded 160 g of a resinous residue, 60 g of which was chromatographed on polyamide sorbent. The column was washed with water and with 10% and 95% ethanols. Recrystallization from 95% ethanol yielded 50 mg of gallic acid (IV) (mp 260°C, M^+ 170). The 10% ethanol eluates were evaporated and chromatographed on silica gel in the chloroform—methanol system. The fraction obtained with 15% methanol was separated on Sephadex LH-20. Chloroform eluted 20 mg of compound (I); 7% methanol in chloroform, 10 mg of (II); and 10% methanol, 45 mg of (III).

Tricin (I). Light yellow acicular crystals with a greenish tinge, mp 280-282°C, composition $C_{17}H_{14}O_7$, M^+ 330, ν_{CO} 1655 cm^{-1}. Maxima in the UV spectrum, nm: MeOH 245 infl., 270, 352; NaOMe 263, 275 sh., 380; NaOAc 275, 385; NaOAc + H_3BO_3 270, 352; $AlCl_3$ and $AlCl_3$ + HCl 278, 305, 364, 394. PMR spectrum in deuteroacetone (ppm): 13.0 (s, 5-OH); 7.36 (s, H-2', 6'); 6.7 (s, H-3); 6.54 (d, 2 Hz, H-8); 6.26 (d, 2 Hz, H-6); 3.96 (s, 6 H, 2 CH_3O).

Tricin 7-O-β-D-glucopyranoside (II). Light yellow crystals, mp. 247-249°C, maxima in the UV spectra, nm: MeOH 270, 350; NaOMe 262, 414; NaOAc 270, 426; NaOAc + H_3BO_3 270, 350; $AlCl_3$ and $AlCl_3$ + HCl 278, 300, 396. PMR spectrum in DMSO (ppm): 13.0 (s, 5-OH); 7.46 (s, H-2',6'), 6.76 (s, H-3); 6.6 (d, 2 Hz, H-8); 6.4 (d, 2 Hz, H-6); 5.1 (d, 7 Hz, H-1"); 4.2-3.3 (m, 6 H of glucose); 3.88 (s, 6 H, 2 CH_3O).

Tricin 5-O-β-D-Glucopyranoside (III). Faintly yellowish crystals with the composition $C_{23}H_{24}O_{12}$, mp 173-177°C (decomp), $[\alpha]_D^{20}$ −117° (0.5, methanol), ν_{CO} 1635 cm^{-1}. Maxima in the UV spectra, nm: MeOH 263, 348; NaOMe 260, 415; NaOAc 270, 316, 368; NaOAc + H_3BO_3 263, 348; $AlCl_3$ 348. PMR spectrum in deuteropyridine (ppm): 7.44 (d, 2 Hz, H-8); 7.37 (s, H-2',6'); 7.04 (d, 2 Hz, H-6); 6.9 (s, H-3); 5.4 (d, 7 Hz, H-1"); 4.5-3.7 (m, 6 H of glucose); 3.86 (s, 2 CH_3O).

Hexaacetate of (III). mp 118-120°C, $[\alpha]_D^{20}$ −71.6° (0.2 chloroform). PMR spectrum in $CDCl_3$: 7.2 (d, 2 Hz, H-8); 7.1 (s, H-2',6'); 7.0 (d, 2 Hz, H-6); 6.7 (s, H-3); 5.5-5.1 (m, 4 H of glucose); 4.3-3.8 (m, 3 H of glucose); 3.92 (s, 2 CH_3O), singlets of two aromatic (2.37, 3 H; 2.35, 3 H; and four [sic] aliphatic (2.14, 3 H; 2.10, 3 H; 2.05, 6 H) CH_3COO groups; in C_6D_6: 7.00 (s, H-2', 6'); 6.84 (br.s, H-6,8); 6.52 (s, H-3); 5.68 (q, 7 and 9 Hz, H-2"); 5.48 (t, 9 and 9 Hz, H-4"); 5.25 (t, 9 and 9 Hz, H-3"); 4.97 (d, 7 Hz, H-1"); 4.22 (m, 2H-6"); 3.51 (s, 2 CH_3O); 3.5 (m, H-5"); singlets of six CH_3COO groups (2.26, 2.03, 1.91, 1.87, 1.82, 173).

SUMMARY

Tricin and its 7- and 5-O-glucosides have been isolated for the first time from the rhizomes of *Rhodiola rosea* L. The physicochemical constants, not previously described in the literature, of tricin 5-O-β-D-glucopyranoside have been determined.

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