Heraclesol Acetonide (III). A solution of 10 mg of heraclesol (I) in 20 ml of anhydrous acetone was treated with 50 mg of anhydrous copper sulfate and the reaction mixture was boiled for 2 h with periodic monitoring of the course of the reaction by paper chromatography in the petroleum ether—formamide system (R_f of the acetonide 0.6). After this, the copper sulfate was separated off, the acetone was distilled off, and the residue (11 mg) was crystallized from ethanol. The acetonide (III) obtained had mp 114-115°C, $[\alpha]_D^{20}$ +10° (c 0.2; methanol) and the empirical formula $C_{20}H_{20}O_7$. The absorption maxima in the UV spectrum corresponded to those of the initial substance.

Methylation of 6-Hydroxy-5-methoxyangelicin (II) to Pimpinellin (IV). Substance (II) (10 mg) was dissolved in 3 ml of a solution of diazomethane in diethyl ether. After the disappearance of the yellow coloration, a solution of diazomethane was added to the reaction mixture dropwise until the yellow coloration had been restored. Then the ether with the remains of the diazomethane that had not reacted was distilled off and the residue was crystallized from ethanol. This gave 7 mg of acicular crystals (mp 148-151°C, empirical formula $C_{13}H_6O_5$), identical with pimpinellin [4].

SUMMARY

The roots of Lesko's cow parsnip have yielded 13 substances of coumarin nature: osthole, psoralen, bergapten, xanthotoxin, isopimpinellin, phellopterin, heracol, biacangelicin, angelicin, sphondin, isobergapten, 6-isopentenyloxy-5-methoxyangelicin, and heraclesol.

Heraclesol is a new compound and is (+)-6-[2(R)3-dihydroxy-3-dimethylbutoxy]-5-methoxy-furo-2',3':7,8-coumarin (I), and this is the first time that 6-isopentenyloxy-5-methoxy-angelicin has been obtained from a plant of the genus *Heracleum*.

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OSCILLOPOLAROGRAPHY OF RUTIN

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Rutin — vitamin P — is a natural flavonoid glucoside. There is information in the literature on the classical polarography of rutin [1, 2]. Oscillopolarography has advantages and greater possibilities.

We have studied the oscillopolarographic behavior of pure rutin under various conditions. We investigated pure rutin with mp 189-191°C on an OP-03 oscillopolarograph with a dropping mercury electrode. The comparison electrode was a saturated calomel electrode. The supporting electrolytes were acetate—ammonium buffer solutions at pH 3-8. The rutin solution was prepared in 96% ethanol. The rutin solution to be polarographed contained, after dilution, 8% of ethanol in a 0.1 N solution of the supporting electrolyte. The measurements were performed with linear and triangular voltage sweeps.

On a support of the buffer solutions mentioned, with an increase in the pH at a constant concentration of rutin a shift in the reduction potential (E_r) in the negative direction was observed:

$-E_r$	1,25	1,35	1,41	1,55	1,60	1,63	1,70	1,80	
pH	3	4	5	6	7	8	9	10	$\frac{\Delta E}{\Delta pH} = 72 mV/pH$

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TABLE 1. Change in I_r and E_r as Functions of the Rate of Voltage Sweeping V at Various Values of pH of the Medium (Rutin, $6.75 \cdot 10^{-5}$ M)

log v	V	pH 3,04	pH 4,05	pH 5,04	pH 6,12	pH 7,05	pH 8,07	pH 9,05	pH 10,04
		I_r E_r	I_r E_r	I_r E_r	I_r E_r	I_r E_r	I_r E_r	I_r E_r	I_r E_r
—0,6	0,25	1,0—1,22	0,8—1,3	0,6—1,35	0,8—1,48	1,00—1,57	0,70—1,60	0,6—1,68	1,30—1,75
—0,3	0,5	1,1—1,25	1,2—1,31	0,9—1,38	0,9—1,49	1,20—1,59	1,2—1,60	0,7—1,69	1,70—1,75
0	1	1,7—1,30	1,8—1,32	1,3—1,38	1,2—1,50	1,60—1,60	1,6—1,62	1,3—1,70	2,10—1,78
0,3	2	3,0—1,32	2,8—1,37	1,9—1,40	1,7—1,50	2,10—1,60	2,2—1,62	1,8—1,68	3,35—1,79
0,6	4	4,8—1,38	4,5—1,41	3,8—1,48	2,5—1,52	2,80—1,62	3,0—1,67	2,7—1,70	4,80—1,79
0,9	8	6,0—1,48	6,6—1,50	5,4—1,50	3,6—1,59	4,00—1,68	4,2—1,70	4,0—1,72	6,0—1,75
1,2	16	9,6—1,60	8,8—1,60	7,2—1,60	5,2—1,68		6,2—1,75	6,4—1,73	
$X = \Delta \log I / \Delta \log V = 0,59$			0,60	0,61	0,53	0,43	0,48	0,58	0,47
$\Delta E = 0,38$			0,30	0,25	0,20	0,11	0,15	0,05	0,0
$\Delta E / \Delta \log V = 0,211$			0,162	0,139	0,133	0,073	0,083	0,028	0,04 0,0 0,027

TABLE 2. Changes in I_r and E_r as Functions of the Delay Period of Feeding the Impulse τ at Various Values of the pH of the Medium (Rutin, $6.75 \cdot 10^{-5}$ M)

Delay		pH 3,04	pH 4,05	pH 5,04	pH 6,12	pH 7,05	pH 8,07	pH 9,05	pH 10,4
log τ	τ	I_r E_r	I_r E_r	I_r E_r	I_r E_r	I_r E_r	I_r E_r	I_r E_r	Tr.
—0,3	0,5	0,7—1,25	0,65—1,32	0,45—1,39	0,70—1,52	0,85—1,60	0,6—1,60	0,5—1,67	
0	1	0,85—1,27	0,80—1,32	0,65—1,40	0,80—1,52	0,85—1,59	0,8—1,60	0,7—1,67	
0,176	1,5	0,90—1,25	1,0—1,32	0,80—1,40	1,0 —1,54	1,0 —1,59	0,9—1,60	0,8—1,67	Tr. Tr.
0,301	2	1,20—1,25	1,20—1,34	0,90—1,40	1,25—1,55	1,3 —1,60	1,0—1,60	1,0—1,67	
0,478	3	1,70—1,27	1,40—1,35	1,20—1,40	1,50—1,57	1,4 —1,59	1,4—1,60	1,2—1,67	
0,602	4	2,45—1,29	1,60—1,35	1,50—1,41	1,80—1,55	1,7 —1,59	1,7—1,62	1,45—1,67	
0,70	5	2,60—1,29	2,0—1,35	1,80—1,41	2,15—1,58	2,2 —1,59	2,2—1,60	1,5—1,68	
0,778	6	2,70—1,29	2,50—1,35	1,90—1,41		2,5 —1,59	2,5—1,62		
0,902	8	3,50—1,29	2,90—1,37	2,5—1,41					
1,00	10								
$X' = \Delta \log I / \Delta \log \tau = 0,58$			0,63	0,62	0,49	0,44	0,58	0,23	

The calculations $X = \Delta \log I / \Delta \log V$ and $X' = \Delta \log I / \Delta \log \tau$ were performed after the plotting of the graphs of these relationships.

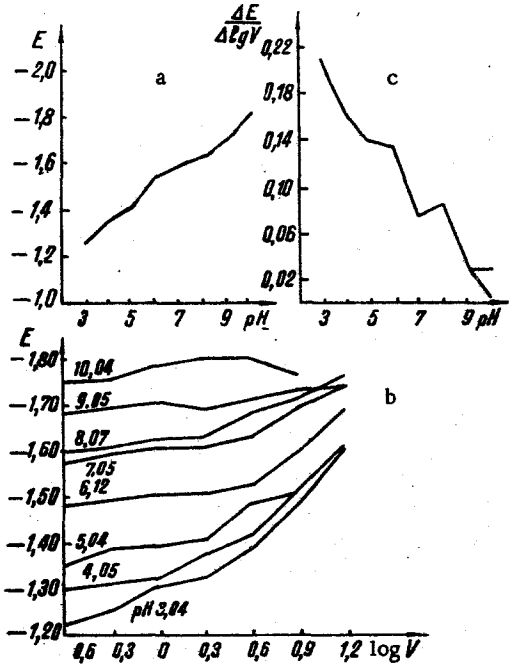

Fig. 1. Dependence of the potential on the pH of a rutin solution (a) and on the rate of voltage sweep at pH 3-10 (b); change in $\Delta E/\Delta \log V$ as a function of the pH of the solution (c). Concentration of rutin in all cases $6.75 \cdot 10^{-5}$ M.

The dependence of E on the pH unambiguously showed the participation of protons in the electrochemical process before the transfer of electrons or simultaneously with it [3].

The shift in potential in the polarography of rutin can be explained by different degrees of protonation of the benzo-γ-pyrone nucleus, by the participation of protons in the electrochemical process of the reduction of rutin, and also by different degrees of ionization of the free phenolic hydroxyls with a change in the pH of the medium. The dependence of E_r on the pH is represented on a graph by a sigmoid curve with several straight-line sections having different slopes, which correspond to the points of the pK values of the reduced and oxidized form for various polarographically active states of rutin (Fig. 1a):

pH	3–4,	4–5,	5–6,	6–8,	8–10	
$\frac{\Delta E}{\Delta \text{ pH}}$	100	60	140	40	85	(mV/pH)

The polarographic peak is most distinct at pH 3-5.

The absence of an anodic peak with triangular sweeping of the voltage at rates of 0.25-16 V/sec, and also the rapid change in potential of the cathodic peak (E_{rc}) in the direction of negative potentials with a rise in the rate of the voltage sweep (V, volts/sec) in an acid medium gives grounds for assuming that the transfer of an electron is followed by a chemical reaction — the dimerization of the electrode products (Fig. 1b, and Tables 1 and 2).

The graphical dependence of $\Delta E_r/\Delta \log V$ on the pH witnesses a change in the nature of the process with a rise in the pH of the solution (Fig. 1c).

The dependence of the peak current (I_r) on the concentration of rutin at pH 3 is shown in Fig. 2c. The nonlinear change in the current can be explained by the adsorption of rutin on the electrode.

The rapid rise in the current with a change in the rate of voltage sweeping V and with an increase in the delay period of the passage of an impulse τ confirms the existence of adsorption of the substance on the electrode at a concentration of $6.75 \cdot 10^{-5}$ M (see Tables 1 and 2).

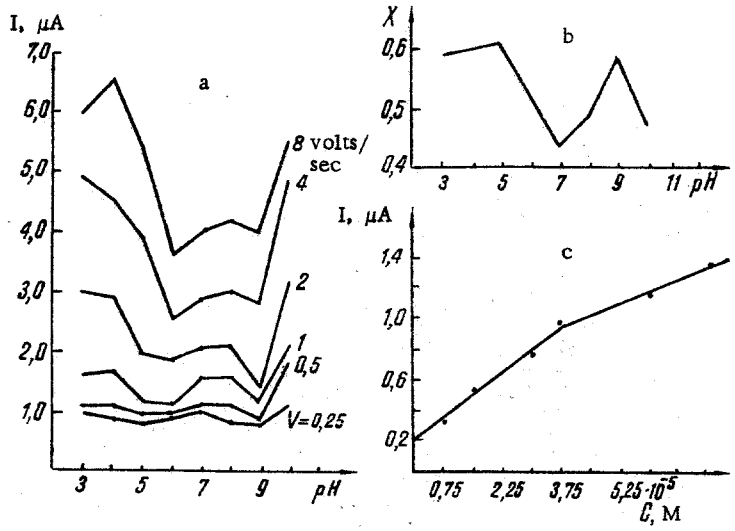

Fig. 2. Dependence of the current on the pH of a solution of rutin at various rates of voltage (a); change in the Semerano coefficient (Δ log I/Δ log V) as a function of the pH of the solution (b) (concentration of rutin in both cases $6.75 \cdot 10^{-5}$ M); dependence of the current on the concentration of rutin (c).

The oscillopolarography of solutions of rutin of the same concentration under similar conditions showed that the current is different at different pH values and the rate coefficient X (Δ log I/Δ log V) has the opposite dependence on the pH of the solution, as can be seen from Fig. 2 a,b and Tables 1 and 2.

The change in the current with a change in the pH of the solutions can be explained by different concentrations of electrochemically active forms of rutin that arise as the result of the dissimilar protonation of the molecules at various pH values and also by the different degrees of ionization of the free phenolic hydroxyls of rutin on passing from an acid to an alkaline medium.

The change in the value of the Semerano criterion (Δ log I/Δ log V) at various pH values can be explained both by a change in the concentration of electrically active forms of rutin and by their different degrees of adsorption on the electrode.

Since for adsorption peaks Δ log I/Δ log V is between 0.5 and 1.0, and I_r is proportional to the concentration (C_o), i.e., $I_r = KC_o$, where $K = 2.344 \cdot 10^5 n^{3/2} D^{1/2} (mt)^{2/3} V^{1/2}$ for reversible electrochemical processes and $K = 2.56 \cdot 10^{-5} n(\alpha n_a)^{1/2} (mt)^{2/3} D^{1/2} V^{1/2}$ for irreversible electrochemical processes [4, 5], on the basis of the results obtained it may be considered that the highest concentration of electrochmically active forms of rutin is observed at pH 3-4, 7-8, and 10 and the lowest concentration at pH 5-6 and 9 (Fig. 2a). In this case, at pH 3-6.2 and 8.2-9.5 there is the phenomenon of adsorption on the electrode, and at pH 6.2-8.2 and 9.5-10.0 the process is controlled by diffusion (see Fig. 2b). Consequently, the greatest sensitivity in the analysis of rutin may be at pH 3-5 with a well-defined adsorption peak.

It is possible to determine rutin from the adsorption peak in acid solutions containing a low concentration of ethanol (8-10%) with great sensitivity and accuracy. But in the analysis of solutions of rutin containing foreign substances adsorbed on the electrode the method gives low results because of the competing absorption on the electrode of the molecules of other surface-active substances. Consequently, to obtain correct analytical results, rutin must be determined under conditions in which adsorption is absent.

It is known that an increase in the concentration of organic solvent in solution decreases the adsorption of substances undergoing reduction.

For rutin, with an increase in the amount of ethanol in the solution from 5 to 30% the peak current decreases threefold and the peak potential shifts in the direction of more negative values. This leads to the fusion of the polarographic peak with the curve of the discharge of hydrogen ions at an alcohol concentration greater than 40%. In order to observe a well-defined peak of the reduction of rutin in the presence of a high concentration of ethanol a buffer solution with a pH of about 6 must be used in order to exclude the discharge of hydrogen ions. With 50% of ethanol in the solution and pH 5, the reduction of rutin has a kinetic nature, as is shown by the sigmoid nature of the polarographic curve and the value of the V criterion, which is 0.29 (pH 5.2). Under these conditions the peak current is directly proportional to the concentration of rutin in the range of concentrations studied — $(0.3\text{-}3.0)\cdot 10^{-4}$M.

To determine rutin in 50% aqueous solution, as model we took tablets of rutin and ascorutin [mixture of ascorbic acid and rutin]. The tablet filler did not interfere with the determination since it scarcely passed into ethanolic solution. Ascorbic acid does not interfere under the given polarographic conditions.

Method of Determination. An accurately weighed sample of powdered rutin tablets equal to the weight of one tablet was placed in a 25-ml measuring flask and shaken with hot 96% ethanol, and the solution was then cooled and made up to the mark. An accurately measured volume of the resulting solution was transferred by a pipette to another measuring flask and ethanol was added in an amount of 50% of the volume of the measuring flask taken, after which the solution was made up to the mark with buffer solution, mixed, poured into the polarographic cell, and subjected to oscillopolarography.

The concentration of rutin in the solution of tablets subjected to polarography was found from a calibration graph of solutions of pure rutin the polarography of which had been performed under the same conditions. The percentage of rutin in the tablets was calculated from the formula

$$X\% = \frac{C_x V_f' V_f'' \cdot 100}{a V_p},$$

where C_x is the concentration of rutin found from the graph, g/ml, V_f' is the volume of the measuring flask for the initial solution, V_f'' is the volume of the measuring flask after the dilution of the solution, V_p is the volume of the pipette, and a is the weight of the sample, g.

The results of analyses of tablets of rutin and ascorutin are given below (according to the factory instructions, the rutin tablets should have contained 8% of pure rutin and the ascorutin tablets 12%):

Number of experiments	Amount of rutin found in the rutin tablets, %	$(\overline{X}-X_i)^2$	Number of experiments	Amount of rutin found in the ascorutin tablets, %	$(\overline{X}-X_i)^2$
$n=6$	$\overline{X}=8,4\%$	$\Sigma=0,65$	$n=6$	$\overline{X}=11,8\%$	$\Sigma=0,6825$
$S=0,65:5=0,36$			$S_n=0,6825:5=0,37$		
$\Delta X=2,57\cdot 0,36:6=0,38$			$\Delta X=2,57\cdot 0,37:6=0,4$		
$\Delta X_{exp\,\%}=0,38\cdot 100:8,4=4,5\%$			$\Delta X_{exp\,\%}=0,4\cdot 100:11,8=3\%$		

SUMMARY

It has been shown that in the oscillopolarography of rutin the electrochemical reduction is affected by the pH of the solution and the concentration of ethanol. Protonation of the rutin molecules and their adsorption on the electrode in aqueous solutions are observed. The adsorption is reduced by increasing the concentration of ethanol to 50%.

The change in current at various pH values with a constant concentration of rutin is due to a change in the concentration of protonated and ionized polarographically active forms of rutin.

Conditions for the quantitative analysis of rutin have been found.

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O-ACYLATED FLAVONOID GLYCOSIDES OF THE NEEDLES OF *Pinus sylvestris*

I. O-ACETYLATED DERIVATIVES OF FLAVONOL GLYCOSIDES

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UDC 547.972

In the field of well-studied flavonoid glycosides, a new group of compounds has appeared relatively recently — their acylated derivatives. The acylating acids are more frequently hydroxyaromatic acids (p-coumaric, ferulic) and more rarely aliphatic acids (acetic, malonic) [1]. The most disputed question has been that of the position of the acyl residue. At the present time it has been established that the following sequence of binding of the fragements exists: heterocycle (flavonoid) — carbohydrate — acid. A similar sequence of binding is known in the field of nitrogen-containing heterocycles (mononucleotides).

There is no information on the presence of O-acylated flavonoid glycosides in coniferous woody plants, with the exception of a recent report of the detection of such compounds in larch needles [2].

From the needles of the Scotch pine (*Pinus sylvestris* L.) we have isolated three compounds (I-III) that are O-acetylated derivatives of flavonoid glycosides.

From the results of hydrolytic cleavage, (I) contains isorhamnetin and glucose, II contains isorhamnetin and galactose. and the components of (III) are quercetin and glucose. Compounds (I-III) differ chromatographically from the corresponding 3-glycosides though they have IR spectra identical with them.

The acetylation of the flavonol glycosides can be judged from their IR and PMR spectra. The IR spectra of compounds (I-III) contain an additional band, as compared with the IR spectra of the corresponding flavonol 3-glycoside, of an acetate carbonyl at 1720 (I), 1730 (II), and 1705 (III) cm^{-1}. The PMR spectra of compounds (I-III) include three-proton singlets at 1.75, 1.61, and 1.70 ppm, respectively, which shows the presence of a CH_3COO group in the carbohydrate moiety of each molecule. This conclusion is also confirmed by the fact that the acetylation of (I) gave an acetate identical with isorhamnetic 3-O-β-D-glucopyranoside heptaacetate; the full acetate of (III) was identical in composition, melting point, and PMR spectrum with isoquercetin octaacetate, and the product of the alkaline saponification of (II) was identical in respect of its UV spectrum and chromatographic behavior with isorhamnetin 3-O-β-D-galactoside [3].

The question of the position of the acetyl residue in the molecule has been solved mainly on the basis of PMR spectra. The PMR spectra of compounds (I-III) in [D]pyridine and of their TMS ethers in CCl_4 contain in the weak field, in addition to the signal of the anomeric proton, two-proton multiplets the origin of which can be explained only by the acetylation of a primary alcohol group of a glucose or galactose residue [4]. If the secondary hydroxyls at C-2", C-3", or C-4" in the molecule had been acetylated, one-proton signals in the form of a triplet or doublet of doublets would have been observed in the weak field. Thus, we

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