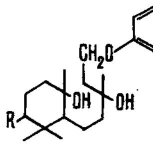
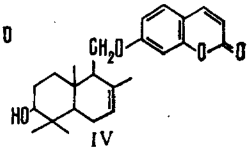
On the basis of the facts presented, for (IV) we propose the empirical structure shown below, which explains the position of the tertiary hydroxy groups in (II), these being split out with the formation of a cyclic product of dehydration.



I. R=OCOCH$_3$
II. R=OH
III. R=O



IV

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NEW METHODS OF IDENTIFYING NATURAL HYDROXYCOUMARINS

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At the present time, hydroxycoumarins are identified by characteristic physical, physicochemical, and chemical properties [1-3]. As compared with alkylated coumarins, they have a stronger fluorescence in UV light [1-3], a reduced sublimability, diffuse absorption bands of the lactone ring in the UV spectra [1], a low value of the integral intensity of the absorption of the carbonyl group of the lactone ring in the IR spectra [1], a characteristic formation of intermolecular hydrogen bonds, ease of acetylation and esterification [2], and other chemical properties.

In an investigation of complex-formation with metal salts and of electrochemical reduction at a dropping mercury electrode (DME), we found a series of new chemical and physicochemical properties of the natural hydroxy coumarins. On electrochemical reduction at a DME of the natural hydroxycoumarins, diffuse polargraphic waves are formed because of the appearance of intermolecular hydrogen bonds. Alkylation and complex formation with metal salts eliminates the diffuseness of the waves and permits the energy of the intermolecular hydrogen bond to be estimated from the shift in the polarographic half-wave potential.

On electrolysis at a DME, solutions of fraxinol are colored the intense lemon yellow that is characteristic for quinoid compounds [4]. There is a linear relationship between the optical density of this coloration and the concentration of the depolarizer (fraxinol), as we showed on an SF-4 instrument. Esculetin gives a similar coloration in the presence of Al_2O_3. The remaining alkyl- and hydroxycoumarins of the 40 compounds that we investigated do not give such a coloration, which can be used for their specific identification.

The inhibiting action of hydroxycoumarins revealed in a reduction of the rate of oxidation of sulfites by molecular oxygen is two to three times greater than for alkylated coumarins. With an increase in the number of phenol groups and of the molecular weight, the inhibiting action rises symbatically. The detection of the reaction was performed by a polarographic method at a DME until the complete disappearance of the maximum of the first oxygen wave [5].

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We and a number of other authors [6] have reported complex formation by hydroxycoumarins and metal salts. This is most strongly shown for esculetin. The complex formation of esculetin in ethanol was studied by spectrophotometric, polarographic, and chromatographic methods with copper, tin, and gold salts. Using copper salts as an example, it was shown that the complex formation of esculetin with metal salts takes place extremely protractedly and in several stages with the formation of a mixture of complexes of different chemical compositions.

Thus, the information given in previous publications [6], the authors of which did not perform a preliminary chromatographic separation of the several possible complexes with one reagent, can be considered doubtful in view of the closeness of the physicochemical properties of the different components of donor–acceptor nature having the same absorption spectrum in the visible region.

By paper and thin-layer chromatography we detected two zones (corresponding to complexes of different chemical structures) in a mixture of ethanolic solutions of esculetin and $CuCl_2$ in concentrations of the same order of magnitude ($2 \cdot 10^{-2}$ M) with R_f values on paper of 0.00 and 0.82 and on silica gel of 0.00 and 0.35, respectively. In UV light, the zones at the start fluoresced brown and the others claret-colored.

The spectral characteristics of the zones were: λ_{max} 385, 310 nm and λ_{min} 280 nm, respectively. Polarographic characteristics: $E_{1/2}$ (at a DME) on a support of 5% LiCl in ethanolic eluates of chromatograms: 0.96 V copper, 1 V esculetin. In its shape, the wave differed sharply from the waves of ethanolic solutions of esculetin and cupric chloride, which shows the existence of complex-formation.

The stoichiometric ratio of copper and esculetin was studied by the method of isomolar series [7], spectrophotometry, and chromatography and was found to be 1 : 2. With tin in alcoholic solutions, esculetin gives a yellow complex (λ_{max} 360 nm) and with gold–hydrochloric acid it gives a black-brown complex.

The polarographic measurements were performed in a thermostatic cell (25°C) on an LP-60 polarograph. Characteristics of the capillary: 1.80 $mg^{2/3} \cdot sec^{-1}$.

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