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Polarography of Coumarins (Chromen-2-ones)

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The mechanism of the reduction of natural and synthetic coumarins at a dropping mercury electrode is examined. The dependence of the half-wave potential on chemical structure is demonstrated by means of correlation equations and quantum-chemical characteristics. The polarographic properties are interpreted in terms of other physicochemical characteristics (optical, adsorption, polarisation, kinetics of important chemical reactions, microwave-spectroscopic, etc.). Polarography can be applied in various fields of the chemistry of coumarins (in the study of adsorption, complex formation, inhibition of chain oxidations, and the mechanism of organic reactions). A list of 200 references is included.

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I. INTRODUCTION

Coumarins are typical heterocyclic compounds, present as structural units in the molecules of tanning agents, antibiotics, phytocides, and a large group of polyphenolic compounds[1-3]. Considerable interest in this group of natural compounds arose from the specificity[1,2] and the breadth of their biological action[3,4]. They have since found wide application in analytical chemistry as luminescent and other indicators[5], optical brightening agents in the textile industry[6], sensitisers in copying techniques[7], aroma-producing substances in foodstuffs, printing, and perfumery industries[1], organic reagents in laser technology[8], toxic chemicals in agriculture[9], and pharmaceutical preparations[1,10] in medicine. In view of the specificity of their physicochemical properties further application of coumarins is envisaged in the production of organic semiconductors, scintillation materials, quantum generators, vitamins, and various organic reagents.

Further investigation of the physicochemical properties of these compounds is of great interest for expanding their fields of application, studying the peculiarities of their electronic structure, elucidating their biological role and aspects of their biosynthesis, and revealing effective new methods of preparation. Polarography has gained a lasting position among other physicochemical methods of examining organic compounds and enables a vast range of theoretical and practical problems to be solved. Results obtained by the polarographic investigation of coumarins can be transferred to other heterocyclic compounds and classes of organic substances.

A considerable number of papers have now appeared on the polarography of coumarins, but there is no critical review of existing achievements in this field with an indication of future lines of research. The present Review discusses not only the polarography of coumarins but also aspects of that of other classes of organic compounds (carbohydrates, cardiac glycosides, chromones, and flavonoid compounds, cinnamic and hydroxycinnamic acids) to the extent necessary for the interpretation of polarographic data and other physicochemical investigations on coumarins.

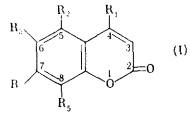
II. MECHANISM OF ELECTRODE REACTIONS

A necessary condition for a heterocyclic compound to be electrochemically active is that the ring should be conjugated and the compound polarisable. The most objective criteria of the presence of conjugation are provided by spectral data characterising the distribution of π-electron density in the molecule and by the dipole moments. Thus several workers have shown[1,11-13] that hydrogenation of the $C_{(3)}=C_{(4)}$ double bond in the 2-pyrone ring of coumarin entails disappearance of fluorescence, characteristic absorption bands, and reducibility at a dropping mercury electrode[14], because of the disruption of conjugation in the pyrone ring. The dipole moment together with the conjugation characteristic indicates also the degree of polarisability of the molecule[15,16]. Dipole moments lying in the range 4.0–8.0 D have been determined experimentally and calculated by an additive vector scheme for several compounds of the coumarin series[16-19]. The dipole moment of coumarin itself has been measured[16] as 4.5 D.

Voitkevich showed[20] that increase in the dipole moment of aliphatic and aromatic compounds is accompanied by increase in their depolarising power at a dropping mercury electrode. However, there may be departures from this general rule, since electrochemical reduction and oxidation depend on many factors and are complicated by adsorption, protonation, dimerisation, solvation, etc. In several cases the dipole moments of the electrochemically active group are more significant than that of the whole molecule. Furthermore, the electrochemical activity of a molecule can be assessed more accurately from the dipole moment not under static conditions, as is usually done, but in an excited state[21].

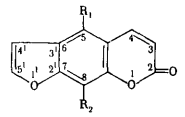
Several workers have shown that both coumarin itself and its derivatives are reduced at a dropping mercury electrode[14,22-60] when various salts—lithium, ammonium, and potassium chlorides, tetraethylammonium iodide and its base, and also Britton–Robinson buffer solutions—are used as supporting electrolyte. Various media have been employed—alcoholic (methanol, ethanol, and propan-1(and 2)-ols), aqueous alcohols, dimethylformamide, and acetone.

The subjects of investigation were natural coumarins of the coumarin (I)



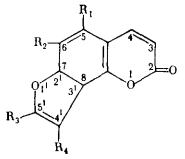
(I)

psoralen (II)



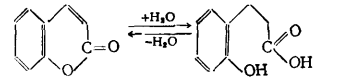
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and angelicin (III)



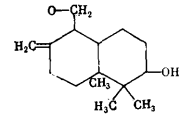
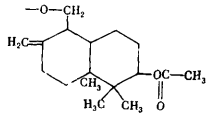
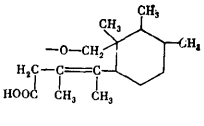
(III)

series, whose principal polarographic characteristics are given in Tables 1–3. These show that coumarin derivatives are reduced at a dropping mercury electrode in two stages, producing in aqueous methanol two waves with tetraethylammonium iodide, and one wave with lithium chloride, as supporting electrolyte. A single wave is obtained also in Britton–Robinson buffer solutions. However, two reduction waves are formed in tetraethylammonium hydroxide used as base in the buffer mixture. The first wave at pH > 10 decreases in height with time, owing to cleavage of the lactone ring[1,33–36,56,62–70]:



The influence of the nature of the base, the substituent, and the solvent on the kinetics of this process has been investigated[56]. Fission of the lactone ring can be affected by daylight and by ultraviolet radiation[1,36].

Table 1. Chemical structure and polarographic constants of compounds of the coumarin series*.

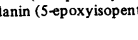
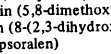
Compound	R_1	R_2	R_3	R_4	R_5	$-E_{1/2}$, V(s.c.e.)**, in $(C_2H_5)_4NI$ I	$-E_{1/2}$, V(s.c.e.)**, in $(C_2H_5)_4NI$ II	$-E_{1/2}$, V(s.c.e.)**, in LiCl I	K_D, *** $\mu A\, s^{1/2}\, mM^{-1}\, mg^{-2/3}$ I	K_D, *** $\mu A\, s^{1/2}\, mM^{-1}\, mg^{-2/3}$ II
Coumarin	H	H	H	H	H	1.40	1.97	1.30	4.20	3.70
6-Methylcoumarin	H	H	CH_3	H	H	1.42	1.92	1.78	4.07	3.50
Umbelliferone (7-hydroxycoumarin)	H	H	H	OH	H	1.47	1.65	1.88	1.30	1.01
4-Methylumbelliferone	CH_3	H	H	OH	H	1.61	1.74	1.74	1.77	1.54
Herniarin (7-methoxycoumarin)	H	H	H	OCH_3	H	1.44	1.94	1.77	4.07	3.50
Coumarin-7-yl acetate	H	H	H	$O-C(O)CH_3$	H	1.35	1.80	1.66	4.00	3.33
7-Isopentenyloxycoumarin	H	H	H	$O-CH_2-CH=C(CH_3)_2$	H	1.51	2.08	1.87	2.98	2.55
7-Geranyloxycoumarin	H	H	H	$O-CH_2-CH=\underset{\displaystyle CH}{\underset{\vert}{C}}H_2-CH_2CH=C(CH_3)_2$	H	1.51	1.86	—	2.32	—
Umbelliprenin (7-farnesyloxycoumarin)	H	H	H	$O(CH_2CH=\underset{\displaystyle CH_3}{\underset{\vert}{C}}-CH_2)_2-CH_2CH=C(CH_3)_2$	H	1.51	2.05	1.84	3.38	3.10
Farnesiferol A (7-(8-hydroxy-6,7,7-trimethyl-3-methylene-[4,4,0]dec-2-ylmethoxy)-coumarin)	H	H	H		H	1.86	—	1.90	1.76	—
Aesculetin (6,7-dihydroxycoumarin)	H	H	OH	OH	H	1.50	1.78	—	0.51	0.73
Aesculin (6-glucosyl-7-hydroxycoumarin)	H	H	$OC_6H_{11}O_5$	OH	H	1.59	1.74	1.70	1.48	2.07
Osthole (8-isopentenyloxy-7-methoxycoumarin)	H	H	H	OCH_3	$CH_2CH=C(CH_3)_2$	1.53	2.08	1.90	4.86	2.92
Fraxinol (6-hydroxy-5,7-dimethoxycoumarin)	H	OCH_3	OH	OCH_3	H	1.53	1.93	1.81	3.37	2.53
Fraxinyl (5,7-dimethoxycoumarin-6-yl) acetate	H	OCH_3	$O-C(O)-CH_3$	OCH_3	H	1.43	1.88	1.70	3.72	3.00
Farnesiferyl acetate	H	H	H		H	1.67	—	—	2.70	—
Galbanic acid (7-(2-(4-carboxy-1,2-dimethylprop-1-enyl)-1,5,6-trimethylcyclohexyloxy)coumarin)	H	H	H		H	1.70	1.84	—	2.00	2.00

* Potential of dropping mercury electrode monitored potentiometrically[61].

** I = first wave; II = second wave.

*** With tetraethylammonium iodide as supporting electrolyte in 75% methanol.

Table 2. Chemical structure and polarographic constants of compounds of the psoralen series*.

Compound	R_1	R_2	$-E_{1/2}$, V(s.c.e.)**, in $(C_2H_5)_4NI$ I	$-E_{1/2}$, V(s.c.e.)**, in $(C_2H_5)_4NI$ II	$-E_{1/2}$, V(s.c.e.)**, in LiCl I	K_D^{***}, $\mu A\ s^{1/2}\ mM^{-1}\ mg^{-2/3}$ I	K_D^{***}, $\mu A\ s^{1/2}\ mM^{-1}\ mg^{-2/3}$ II
Psoralen (furo[3,2-*g*]coumarin)	H	H	1.44	1.95	1.83	3.1	2.30
Bergaptol (5-hydroxypsoralen)	OH	H	1.43	—	—	—	—
Bergapten (5-methoxypsoralen)	OCH_3	H	1.45	1.86	1.85	3.34	2.85
Isoimperatorin (5-isopentenyloxypsoralen)	$O-CH_2CH=C(CH_3)_2$	H	1.45	—	—	—	—
Oxypeucedanin (5-epoxyisopentenyloxypsoralen)		H	1.67	2.00	1.73	3.42	2.76
Xanthotoxol (8-hydroxypsoralen)	H	OH	1.50	2.00	1.79	3.20	2.38
Xanthotoxin (8-methoxypsoralen)	H	OCH_3	1.38	1.85	1.66	4.12	3.57
Xanthotoxyl (psoralen-8-yl)acetate	H	$O-C(O)-CH_3$	1.35	—	—	1.01	—
Imperatorin (8-isopentenyloxypsoralen)	H	$O-CH_2-CH=C(CH_3)_2$	1.42	1.35	1.80	3.05	2.90
Isopimpinellin (5,8-dimethoxypsoralen)	OCH_3	OCH_3	1.45	1.81	1.79	—	—
Biacangelicin (8-(2,3-dihydroxy-3-methylbutoxy)-5-methoxypsoralen)	OCH_3		1.41	1.93	—	2.00	1.80
Cnidilin (5-isopentenyloxy-8-methoxypsoralen)	$O-CH_2-CH=C(CH_3)_2$	OCH_3	1.45	1.91	1.81	—	—
Cnidicin (5,8-di-isopentenyloxypsoralen)	$O-CH_2-CH=C(CH_3)_2$	$O-CH_2-CH=C(CH_3)_2$	1.41	1.86	1.78	—	—
Alloimperatorin (8-hydroxy-5-isopentenylpsoralen)	$CH_2-CH=C(CH_3)_2$	OH	1.56	1.80	—	1.37	1.30

* Potential of dropping mercury electrode monitored potentiometrically[61].
** I = first wave; II = second wave.
*** With tetraethylammonium iodide as supporting electrolyte in 75% methanol.

Table 3. Chemical structure and polarographic constants of compounds of the angelicin series*.

Compound	R_1	R_2	R_3	R_4	$-E_{1/2}$, V(s.c.e.)**, in $(C_2H_5)_4NI$ I	$-E_{1/2}$, V(s.c.e.)**, in $(C_2H_5)_4NI$ II	$-E_{1/2}$, V(s.c.e.)**, in LiCl I	K_D^{***}, $\mu A\ s^{1/2}\ mM^{-1}\ mg^{-2/3}$ I	K_D^{***}, $\mu A\ s^{1/2}\ mM^{-1}\ mg^{-2/3}$ II
Angelicin (fuoro[2,3-*h*]coumarin)	H	H	H	H	1.48	1.89	1.80	2.41	1.50
Isobergapten (6-methoxyangelicin)	H	OCH_3	H	H	1.55	1.94	1.86	2.70	2.20
Sphondin (5-methoxyangelicin)	OCH_3	H	H	H	1.45	1.90	1.88	4.12	3.57
Pimpinellin (5,6-dimethoxyangelicin)	OCH_3	OCH_3	H	H	1.53	2.08	1.81	2.20	2.00
Oreosolone (5'-isopropenylangelicin)	H	H		H	1.47	1.94	1.82	1.61	1.50
Oreosolol (5'-(1-hydroxy-1-methylethyl)-angelicin)	H	H		H	1.48	—	—	2.20	—
Methyl oreosolyl ether (5'-(1-methoxy-1-methylethyl)angelicin)	H	H		H	1.48	—	—	1.89	—
Peucenidin (5'-(1-acetoxy-1-methylethyl)-4',5'-dihydro-4'-senecioylangelicin)	H	H			1.43	1.99	1.79	0.3	0.90
Athamantin (4',5'-dihydro-4'-isovaleryl-oxy-5'-(1-isovaleryloxy-1-methylethyl)-angelicin)	H	H			1.60	2.12	1.77	1.34	1.00
Libonatin (4'-acetoxy-4',5'-dihydro-5'-(2-methylbut-2-enoyl)angelicin)	H	H		$O-C(O)-CH_3$	1.47		1.80	1.02	3.40

* Potential of dropping mercury electrode monitored potentiometrically[61].
** I = first wave; II = second wave.
*** With tetraethylammonium iodide as supporting electrolyte in 75% methanol.

Investigation of the kinetics and the mechanism of cleavage of the lactone ring not only is of theoretical interest in connection with the chemistry of lactones, on whose physicochemical and kinetic characteristics very few data are available, but also is one of the most important aspects of the chemistry of coumarins, since a considerable number of their fundamental chemical reactions (hydrogenation, azo-coupling, methylation, oxidation,

halogenation, mercuration, and arsenation) are based on preliminary cleavage of the lactone ring in alkaline medium and are widely used for the identification[1-3,68,71-72], quantitative analysis[71-73], preparative isolation[1,73], and synthesis[68,73] of these compounds. Experimental results confirm that the lactone ring opens in alkaline medium. The initial wave of coumarin is obtained when the solutions are acidified to neutral pH[14,36]. Reaction velocities at high rates of cleavage of the lactone ring are determined by oscillographic polarography[35].

The morphology of the waves from coumarin derivatives and their relative heights exhibit several special features. Waves due to hydroxycoumarins are diffuse, showing a considerable tendency to coalesce owing to the small differences in half-wave potentials caused by the formation of intermolecular hydrogen bonds. The hydroxycoumarins—fraxinol and aesculetin—on polarographic reduction give an intense lemon-yellow coloration, the optical density of the solutions remaining constant for a long time[14]. Spectral investigations have shown that the formation of coloured products is due to conversion of the coumarin into a quinonoid system. Only one wave is recorded with farnesiferol, clearly owing to the impossibility of obtaining the second stage of reduction in tetraethylammonium iodide as supporting electrolyte. With the acylangelicins—peucenidin and libanotin—the second wave is threefold the height of the first wave, as a consequence of further reduction at the second stage of residues of organic acids present in side-chains. These features together with the considerable differences among half-wave potentials for several coumarins—coumarin itself, aesculetin, umbelliprenin, farnesiferol, psoralen, xanthotoxol—may form a basis for the identification and qualitative analysis of coumarins.

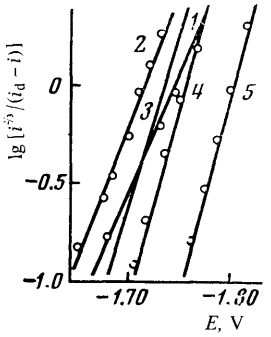


Figure 1. Dependence of $\lg[i^{2/3}/(i_d - i)]$ on E for the first wave of coumarin in 3% tetraethylammonium iodide in methanolic (vol.%) solutions: 1) 12.5; 2) 25.0; 3) 37.5; 4) 62.5; 5) 87.5.

The linear concentration dependence of the limiting current[14,27-30,35-39,41-60] and also the temperature coefficients (1.2–2.0% deg^{-1}) indicate a limiting diffusion current of coumarin derivatives when they are reduced at a dropping mercury electrode. These relationships have been verified with various supporting electrolytes (lithium chloride, tetraethylammonium iodide, Britton–Robinson buffer) in aqueous, alcoholic (methyl, ethyl, propyl, and isopropyl), and aqueous–alcoholic solutions, in dimethylformamide alone and mixed with water and with alcohols, and in acetone. With the quaternary ammonium iodide a linear concentration dependence of the limiting current has been established[14] for both reduction stages in the concentration range 0.1–0.001 mass %, which formed the basis for the development of methods for the quantitative analysis of coumarins in various materials[35,41-57,60]. The observed limiting currents are consistent with the Ilkovič equation[74].

Čapka[27] used the diffusion coefficients of acetophenone and benzophenone in comparing the limiting currents, and reached the erroneous conclusion that coumarins undergo two-electron reduction at the potential of the first wave.

Polarographic coulometry[75], with graphical analysis of the voltage–current curves by means of the Heyrovský[76] and Mairanovskii[77] equations, has shown that the first stage in the reduction of coumarins in various supporting electrolytes takes place reversibly, with addition of a single electron followed by slow dimerisation (Fig. 1). Cyclic polarograms obtained at a suspended drop also show reversibility of the electrode process. The half-wave potential becomes more negative with increase in the alcohol concentration. Fig. 1 shows that this involves little change in the reversibility.

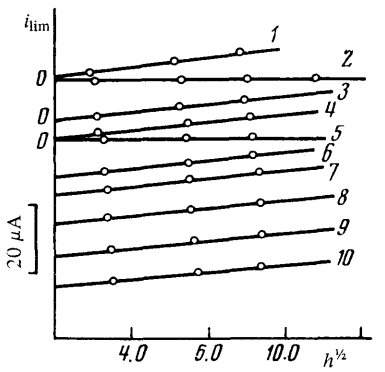
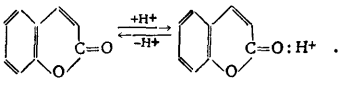


Figure 2. Dependence of limiting current of first wave of coumarin on $h^{1/2}$ in solutions of ionic strength 0.39 at different pH-values: 1) 3.29; 2) 3.79; 3) 4.10; 4) 4.50; 5) 5.02; 6) 7.10; 7) 8.36; 8) 8.91; 9) 10.36; 10) 11.2.

The half-wave potential at the first reduction stage remains unchanged over the pH range 6–9. In the second stage, recorded only in Britton–Robinson buffer solutions containing tetraethylammonium hydroxide as base, the half-wave potential depends on the pH, which can be explained by polarography of cinnamic and hydroxycinnamic[78-80] acids, in whose reduction the dissociation of their phenolic and carboxylic groups plays a considerable part[81]. Therefore the first and second half-wave potentials of hydroxycoumarins change with increase in pH. The second stage in the cathodic reduction of still undecomposed coumarin in the presence of tetraethylammonium hydroxide is accompanied by reduction of hydroxycinnamic acids formed by cleavage of the lactone ring in the coumarins. The second wave is therefore complicated in character. The half-wave potential of hydroxycinnamic acids, like that

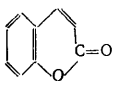
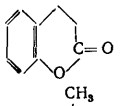
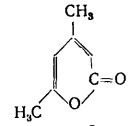
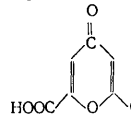
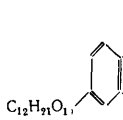
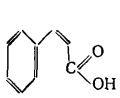
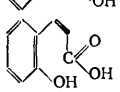
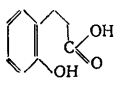
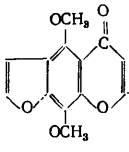
of cinnamic acids[76,82-88], is markedly dependent on pH, and hence that of the second wave of coumarins also depends on pH under the above conditions.

The occurrence of kinetic currents at acid pH values (Fig. 2, curves *2* and *5*), the variation of the half-wave potential over the pH range 4.1–6.0, the electronic structure, quantum-chemical calculations[89], the basicity constants[90], and also the mechanism of formation of oxonium salts[91-93] prove that coumarin molecules undergo protonation:



Thus coumarins exhibit a diffusion current at pH 4.10–10.36; a coumarin wave cannot be recorded at pH < 3.29, owing to superposition of the discharge of hydrogen ions on the reduction of coumarin. Investigations of the dependence of I_d on $h^{1/2}$ at various pH[14] indicate a kinetic current over the pH range 3.29–4.10, whose magnitude is determined by the rate of protonation of coumarin. In alkaline medium (pH > 10.3) the strength of the current is limited by the rate of cleavage of the lactone ring. The second reduction wave of coumarins and the wave of cinnamic acids are not recorded at all in supporting electrolytes that are more easily reduced than are tetra-alkylammonium salts, whence Knobloch[28,29] wrongly concluded that cinnamic acids are polarographically inactive.

Table 4. Half-wave potentials of model compounds polarographed in 75% methanol*.

Compound	Structural formula	$-E_{1/2}$, V(s.c.e.), in					
		3% $(C_2H_5)_4NI$		3% LiCl		Britton-Robinson buffer	
		I	II	I	II	I	II
Coumarin		1.40	1.97	1.32	—	1.67 (pH 7.10)	—
3,4-Dihydrocoumarin		—	—	—	—	—	—
4,6-Dimethyl-2-pyrone		1.72	1.94	1.90	2.20	—	—
Chelidonic acid		1.40	1.96	—	—	1.50 (pH 9.15)	—
Hesperedin		1.80	—	—	—	—	—
Cinnamic acid		1.38	1.85	1.67	—	—	—
cis-o-Hydroxycinnamic acid		1.40	1.97	1.99	—	—	—
trans-o-Hydroxycinnamic acid		1.44	1.89	1.86	—	—	—
Kellin**		1.56	2.10	1.57	1.80	—	—

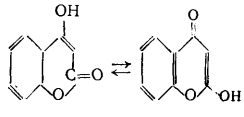
*Dihydrocoumarin was prepared by hydrogenating coumarin in alkaline medium with molecular hydrogen in the presence of a palladium catalyst[94,95]; 4,6-dimethyl-2-pyrone was synthesised from acetoacetic ester[68]; and chelidonic acid was obtained by N. F. Komissarenko from Far Eastern lily of the valley (*Convallaria keiskei Mig.*) in the Phytochemistry Laboratory of the Kharkov Chemical and Pharmaceutical Scientific Research Institute.

**With 3% tetraethylammonium iodide as supporting electrolyte kellin has a third wave with $E_{1/2}$ = -2.35 V.

Various model compounds have been examined[14] in order to interpret the mechanism of polarographic reduction of coumarins at a dropping mercury electrode. The results (Table 4) show that polarographic activity is due to the presence of the conjugated chain CH : CH.CO.O in the 2-pyrone structure. This chain disappears on hydrogenation of the $C_{(3)} : C_{(4)}$ double bond, in contrast to chromones, which have an O : C.CH : C.O chain in the 4-pyrone structure. Coumarins containing substituents having double bonds that are either isolated from or only slightly conjugated with the 2-pyrone ring (farnesan in umbelliprenin, isopentenyl in imperatorin and osthole, ester groups in athamantin) do not show the enhanced electrochemical activity of these groups observed in peucenidin and libanotin.

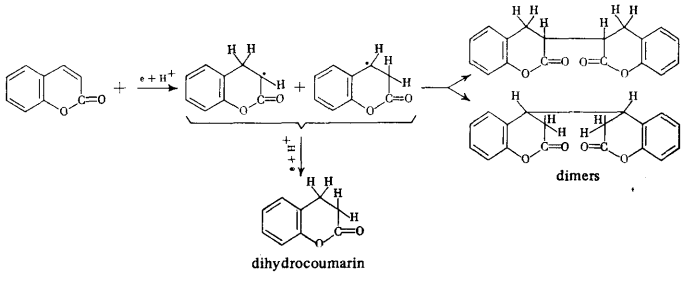
Chromatographic, mass-spectral and spectral (ultraviolet and infrared), and combined chromatographic and mass-spectrometric examination of the products of electrolysis at a dropping mercury electrode and at a controlled potential on a macroelectrode[14], as well as preparative electrolysis[36,39], show that the electrode reaction results in formation of dihydrocoumarin and dimers of coumarin. Other compounds have also been detected in preparative electrolysis, which may be products of hydrogenation by hydrogen[96,97] formed during electrolysis and of subsequent reaction of the products with radicals and with the electrode material[98].

In contrast to chromones and chalcones, coumarins exhibit intense infrared absorption bands due to the carbonyl group[1,11,13] and diminished reactivity towards cationic reactants[68], so that it may be concluded that this group is more weakly protonated in coumarin derivatives than in chalcones[22,23,99-102], flavonoid compounds[22,23,103-111], and chromones[22,23,104-112], whose half-wave potentials depend on the pH and which produce a catalytic current[28,29,77]. Such a current has been observed[43,49] with kellin, a pharmaceutical preparation. Chromones are genetically so close to coumarins in chemical structure that conversion of 4-hydroxycoumarins into 2-hydroxychromone is observed[29]:

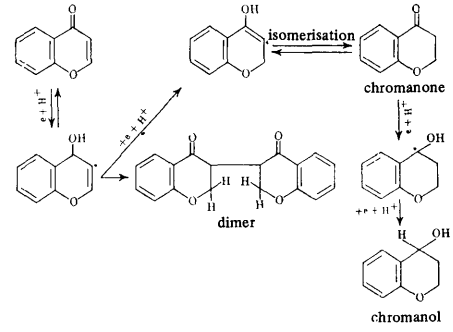


Reducibility at a dropping mercury electrode is observed also with other classes of compounds containing a 2-pyrone ring (cardiac glycosides)[113-117].

The results of the investigations enable schemes to be postulated for reduction at a dropping mercury electrode—for coumarins



and for chromones



The same mechanism of reduction has been suggested by Stradyn'[23] and by Perrin[22] in fundamental reviews on the polarography of heterocyclic organic compounds.

Carbohydrates present in side-chains in coumarins, cardiac glycosides, flavonoid compounds, and chromones are not reduced at a dropping mercury electrode, since their reduction requires the presence of a carbonyl form, which cannot be produced in solution owing to the absence of ring–chain tautomerism in the glycosidic state[24,76]. In the case of furochromones[111,112] (kelloglycoside) the introduction of the carbohydrate residue converts a single-wave into a two-wave polarogram. The polarograms of kellin and the glycoside in various supporting electrolytes are quite complicated, containing diffusion and catalytic waves. In acid medium the electrode process is complicated by formation of protonated complex compounds (oxonium salts), and in alkaline medium by cleavage of the 4-pyrone ring, so that kinetic currents may also be observed.

The reduction of coumarin derivatives at a dropping mercury electrode is accompanied by adsorption phenomena[14,32,118-120] depending on the structure of the electrical double layer[17,32,120].

III. CHEMICAL STRUCTURE AND HALF-WAVE POTENTIAL

One of the most important tasks of the polarography of organic compounds is to find relations between chemical structure and half-wave potential, since knowledge of this correlation will enable the electrochemical activity of organic compounds to be assessed, to a certain extent the mechanism of electrochemical processes, their kinetics, the structure of the products of electrolysis, and also several important physicochemical constants (ionisation potential, electron affinity, redox potential of biochemically important systems), defined directly or indirectly in terms of the half-wave potential, which have played some part in assessing the results of quantum-chemical calculations on the molecules of benzenoid aromatic compounds[121-123]. Such relations can be gauged in several ways—(i) by qualitative and semi-quantitative comparison of half-wave potentials with the electronic structure of functional groups, with conjugation, steric, and inductive effects[24,25,64,76,124], (ii) by means of correlation equations[125-129], (iii) by quantum-chemical methods[121-123,130-131], and (iv) from the relation between the half-wave potential and other physicochemical constants (refraction, dipole moments, ultraviolet and infrared spectra, mass spectra, and microwave spectroscopy—nuclear magnetic and electron spin resonance) containing considerable information on the chemical structure of the molecules[25,74,121,132]—on which

several reviews have been published[25,121,132-136] besides individual chapters in special monographs[74,130,131], devoted to a detailed examination of the relation between half-wave potentials and chemical structure.

In order to trace a correlation with the structure of coumarins and to reach conclusions on the influence of substituents on the half-wave potential, the Reviewer has selected series both of naturally occurring compounds and of products of chemical reactions[40]. Such comparison cannot be based on published values, since half-wave potentials have been obtained by different workers under different conditions (different supporting electrolytes, solvents, and apparatus). The individual values may be contradictory even under the same conditions, and several compounds when tested did not exhibit any polarographic activity (derivatives of 4-hydroxycoumarins)[37,38].

Table 1 gives the chemical structure and the half-wave potentials of derivatives of coumarin, psoralen, and angelicin. Only the values for the first wave, corresponding to a reversible process, were used for the comparison. Table 1 shows that alkylated coumarins, containing donor substituents, are reduced with greater difficulty than are the corresponding unsubstituted compounds. The 2-pyrone ring in the coumarin system is reduced more readily than is 2-pyrone itself, because of conjugation with the aromatic benzene system.

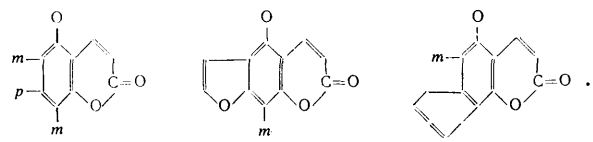
The fusion of a furan ring with the coumarin system in psoralen and angelicin derivatives hinders reduction of the furocoumarins owing to the donor influence of the furan heteroatom. Introduction of ester substituents lowers the half-wave potential of the corresponding hydroxy-compounds (umbelliferone, fraxinyl, xanthotoxyl, and peucenidinyl acetates, libanotin, and athamantin). Coumarin ethers exhibit the considerable electron-donating activity of the radicals, reflected in more negative half-wave potentials. The lengthening of the unconjugated chain in geranyl- and 7-farnesyl-oxycoumarins produces no change in the half-wave potential of 7-isopentenyloxycoumarin. This proves the absence of steric hindrance by groups to reduction of the $C_{(3)}:C_{(4)}$ double bond in benzo-2-pyrone.

Among simple coumarins the highest reduction potential is shown by farnesiferol A, probably because of the specific conformation of the farnesyl group, causing steric hindrance to reduction of the $C_{(3)}:C_{(4)}$ double bond at a dropping mercury electrode[124,137,138]. The half-wave potentials of 5-mono- and 5,8-di-substituted psoralen derivatives lie in the narrow range from −1.41 to −1.45 V, with the exception of xanthotoxol and alloimperatorin, in which the 8-hydroxyl produces a displacement of electron density owing to conjugation with the carbonyl of the 2-pyrone through the heteroatomic oxygen. Angelicin derivatives substituted at position 5 or in the furan ring have values between −1.43 and −1.48 V; the 6-substituted derivatives have more negative half-wave potentials.

Correlation equations have been used to find a quantitative relation with chemical structure, and half-wave potentials have been compared with the energy of the lowest vacant level. Naturally occurring coumarins contain substituents mainly in the benzene ring, at a considerable distance from the reactive centre—the $C_{(3)}:C_{(4)}$ double bond—that undergoes electrochemical hydrogenation. It is therefore advisable to use the Hammett equation, representing the inductive and mesomeric influence of substituents[22,125-129,139,140], whose application has been described in detail by Zuman[127,128]. According to its physical meaning the Hammett equation is applicable mainly to benzenoid compounds, but several workers have shown that it can be used for heterocyclic compounds[22,23,126,141]. Its application requires accurate knowledge of the position of a substituent with respect to the reaction centre, a problem that is made more difficult by the location of substituents and centre in different rings.

In one paper Zuman[58] stated that the Hammett equation must be used for coumarin derivatives substituted in the benzene ring, but the Taft equation[125,126,139] for those substituted in the pyrone ring; yet no information is available on how to control the position of a substituent. Several workers have shown that the orienting effects of substituents depend on the electron density on the carbon atoms[130,142,143].

Published molecular diagrams of coumarin, psoralen, and angelicin[12,89,131] and those calculated by the Reviewer using the LCAO-MO method to the Hückel approximation suggest that the orientation of substituents can be represented by



The test compounds cannot be regarded as belonging to a single reaction series, for they are based on different structures—coumarin, angelicin, and psoralen—although all the compounds are reduced at a dropping mercury electrode by the same mechanism. Furthermore, the influence of a substituent in a given ring changes with its orientation relative to the heteroatoms. In view of these considerations, some of the natural coumarins having tabulated values of σ were divided, on treatment of their half-wave potentials, into the reaction series

(*a*) coumarin, 6-methylcoumarin, umbelliferone, 4-methylumbelliferone, herniarin, coumarin-7-yl acetate

(*b*) aesculin, aesculetin, fraxinol, fraxinyl acetate

(*c*) psoralen, xanthotoxol, xanthotoxin, xanthotoxyl acetate.

For other compounds either tabulated values of σ are not available or the Hammett equation is inapplicable.

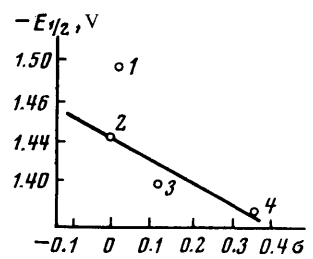


Figure 3. Correlation of half-wave potentials of reaction series (*a*) with Hammett σ constants for: 1) *p*-OH; 2) *p*-OCH_3; 3) *m*-CH_3; 4) H; 5) *p*-$OCOCH_3$.

The results plotted in Figs. 3 and 4 show that reaction series (*a*) and (*c*) satisfy the Hammett equation. Departures from the correlation can be explained by absence of additivity, between the electrochemical activity of the coumarins at the dropping mercury electrode and the σ values, resulting from intra- and inter-molecular hydrogen bonding, adsorption, dimerisation, and solvation effects.

The Reviewer has assessed the rigour with which the Jaffé correlation[125,126,144] is satisfied only on reaction series (*a*), for which the principal correlation parameters have the values $\eta = 0.974$, $\rho = 0.13$, and $S = 0.058$. The positive value of ρ indicates that the reaction is electrophilic in character: i.e. the introduction of electron-donating substituents retards hydrogenation of the double bond, whereas electron-accepting substituents accelerate it (respectively more and less negative half-wave potentials). The small ρ for series (*a*) indicates the low polarity of the transition state. Values of σ have been taken from the paper by McDaniel and Brown[145].

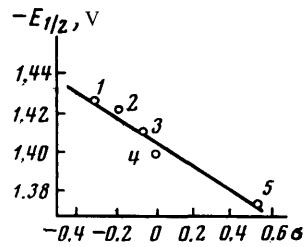


Figure 4. Correlation of half-wave potentials of reaction series (*c*) with Hammett σ constants for: 1) *m*-OH; 2) H; 3) *m*-OCH_3; 4) *m*-$OCOCH_3$.

Coumarin has 11 molecular orbitals, of which 6 are bonding and 5 antibonding. All 12 π-electrons can be distributed among bonding molecular orbitals to form a closed system. The energies of delocalisation and of aromatisation for coumarin are respectively 3.36β and 0.575β†. Of the 14 molecular orbitals in the psoralen molecule 8 are bonding and 6 are antibonding; the 16 mobile π-electrons of psoralen also occupy all the bonding orbitals and form a closer electron system. Thus the compounds studied form closed electron shells with 8, 12, and 16 π-electrons. The Hückel rule is inapplicable to them, since the 2-pyrone ring is not a regular polygon owing to the presence of the carbonyl group, and the $4n + 2$ π-electrons rule is not satisfied.

The polarographic activity of organic compounds involves the donation of electrons from the highest occupied level and the acceptance of electrons by the lowest vacant level, so that the half-wave potential is a linear function of the energy of these levels, and can be measured by the electron affinity or the ionisation potential[130-132,146]. This provides a ready explanation of the satisfactory correlation of this potential with various physical and chemical[74,131,147-152] properties of organic compounds.

A theoretical basis for the possibility of correlating half-wave potentials with the energy of the lowest vacant orbital was first provided by Maccoll[153]. This dependence for the coumarin, psoralen, and angelicin series (Fig. 5) shows that half-wave potentials may be predicted for furocoumarins. Departures from this dependence exhibited by acetylated derivatives and diols (aesculetin) in the coumarin series can be explained by insufficient allowance in quantum-chemical calculations for exchange interaction between adjacent groups and interaction of groups with the solvent or by distortion of the potentials by adsorption, solvation, protonation, and dimerisation of intermediate products.

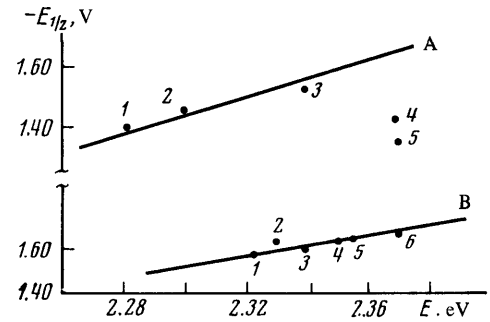


Figure 5. Correlation of energies of lowest vacant orbitals *E* and the polarographic half-wave potentials for the coumarin series (*A*): 1) coumarin; 2) umbelliferone; 3) fraxinol; 4) fraxinyl acetate; 5) umbelliferone acetate; and for the psoralen and the angelicin series (*B*): 1) psoralen; 2) angelicin; 3) xanthotoxin; 4) sphondin; 5) begapten; 6) isopimpinellin.

IV. PHYSICOCHEMICAL PROPERTIES AND HALF-WAVE POTENTIAL

It is of great theoretical and practical interest to find the interrelationship between the polarographic characteristics and the physicochemical properties of organic molecules, for the reasons outlined below.

1. It will become possible to assess the relation between the half-wave potential and chemical structure—more precisely to understand how this potential reflects features in the electronic structure of molecules (inductive and mesomeric effects), conformational characteristics (steric effects), the distribution of electron density, the polarisability and polarity of molecules, electrochemical activity, reactivity, and in several cases also the biological action of the substance.

2. Comparison of half-wave potentials with redox potentials will give information on the reversibility of an electrode process, and sometimes polarography may become an independent method for determining redox potentials, since it is more sensitive than the potentiometric method and can be applied over a wider composition range of the medium (quinones, vitamins).

3. Comparison of half-wave potentials with spectral characteristics will permit estimation of the degree of homolysation of molecules[154-156] and the parameters in the equation relating these potentials with the energy of the orbitals, with some indication of the mechanism of electron transfer in the electrochemical step.

4. Comparison of half-wave potentials with dipole moments and refractions enables the electrochemical activity of individual functional groups or structural units of the molecule to be estimated, and also the direction of the polarisation vector to be assessed.

†Here β represents the resonance integral used in quantum-chemical calculations[130].

5. The relation between the diffusion-current constant (from the Ilkovič equation) and the molecular mass enables the latter to be evaluated, and also to some extent the degree of sphericity of the molecule (which occurs in the main condition for the Stokes–Einstein equation to be satisfied).

6. Comparison of half-wave potentials for the oxidation of organic compounds with mass-spectral characteristics (ionisation potential, peak appearance potential) will enable the ionisation potential to be evaluated.

7. Comparison of the half-wave reduction potentials with the energy of the longer-wavelength band of the donor (charge-transfer complex) will enable the electron affinity to be evaluated.

8. Comparison of half-wave potentials for polarographic oxidation and reduction will enable the alternance and non-alternance of hydrocarbons to be assessed.

Undoubtedly we could still find quite a few important aspects of the theoretical and practical applicability of the correlation of polarographic characteristics with other physicochemical characteristics of the molecules.

The Reviewer has compared half-wave potentials for the polarographic reduction of coumarins with (i) the energy of the longer-wavelength ultraviolet absorption band, (ii) the dipole moments, (iii) mass-spectral characteristics, (iv) the chemical shift in the nuclear magnetic resonance spectra, and (v) the electron affinity, besides comparing the diffusion-current constants in the first wave of coumarins in 5% tetraethylammonium iodide solution as supporting electrolyte with the molecular mass.

1. Half-wave Potential and Ultraviolet Spectra of Coumarins

Comparison of half-wave potentials with ultraviolet absorption band energies is based on the identity of primary physicochemical steps in an electrochemical reaction and in photoexcitation. Watson and Matsen[157]‡ used Maccoll's relation[153] to show that for many compounds the dependence of the half-wave potential on the energy of transition of an electron from the highest occupied to the lowest vacant orbital can be established directly:

$$-E_{1/2}={}^1/_2\,h\nu+\text{const.},$$

where ν is the frequency of the longer-wavelength absorption band.

A defect in most papers in this field is non-observance of the dimensions of the units in which $E_{1/2}$ and $\lambda_m(\nu)$ are expressed. The former is measured in *volts*, and the latter in *nanometres*. If both parameters are expressed in *electron-volts*, the values of $E_{1/2}$ will partly take into account also the mechanism of reduction at the dropping mercury electrode, since $E_{1/2}$ will have to be multiplied by the number of electrons involved in the electrochemical step.

Grabovskii[157] showed that the relations between the reduction potential and the energy of the electron transition may assume different forms depending on the nature of the substituent and the electron transition. There may be other approaches to a physical explanation of the correlation between half-wave potential and ultraviolet spectral data.

Several substances undergoing an electrochemical reaction at a dropping mercury electrode form charge-transfer complexes with components of the supporting electrolyte and act as acceptors, so that the relation[158]

$$h\nu=I_D-E_A+C$$

holds for them, where $h\nu$ (in eV) is the energy of charge transfer, I_D (eV) the ionisation potential of the donor, E_A (eV) the electron affinity of the acceptor, and C a constant. As several workers have shown, a linear dependence exists between $h\nu$ and E_A, as between $h\nu$ and I_D, and since the reduction half-wave potential of an organic molecule most often corresponds to E_A,[159] this potential should vary linearly with the energy of the longer-wavelength absorption band. On the other hand, the frequency of variation in the variable dipole moment P is described by the formula[160]

$$P=P_0\cos(2\pi\nu t),$$

(in which P_0 is the constant dipole moment of the molecule, ν the frequency of vibration of the molecule, and t the time), which shows that the frequency depends on the dipole moment. In several cases the half-wave potential is a linear function of the dipole moment[20,74], so that a linear relation should exist between this potential and the energy of the corresponding absorption band.

The spectrum of coumarins contains 4–6 strong absorption bands (molar extinction coefficients 3000–30 000). The bands are due to $\pi-\pi^*$ transitions[11,12], but $n-\pi^*$ transitions may also occur (masked by $\pi-\pi^*$ transitions). The polarographic reduction of naturally occurring coumarins at a dropping mercury electrode is due, by analogy with other organic compounds, to the presence of a system of delocalised π electrons in the 2-pyrone ring, and is accomplished by passage of electrons from the electrode to the lowest vacant level in the molecule.

The long-wavelength bands corresponding to $\pi-\pi^*$ transitions characterise the energetic state of the functional part of the molecule (containing the conjugated π-electron system), which is also electrochemically active, so that it is the energy of such a band that must be compared with the half-wave potential. The ultraviolet band energies of coumarins were obtained (in electron-volts) from the corresponding λ_m values by means of the classical Bohr frequency rule $h\nu = hc/\lambda = \epsilon_2 - \epsilon_1$.[160] Relative energies of polarographic reduction of the molecules of coumarins ϵ can be determined as the product of the polarographic half-wave potential $E_{1/2}$ and the number of electrons n taking part in the electrochemical step. The half-wave potentials of the first, reversible wave are usually taken in finding the correlation of spectral and polarographic characteristics, as in the present case.

A linear dependence of the half-wave potential of the first wave on the longer-wavelength ultraviolet absorption maximum has indeed been established for the series—coumarin, psoralen, and angelicin—with the potential falling as the band energy increases[41]. However, since the correlation is based on the energy of the whole band corresponding to $\pi-\pi^*$ transitions predominantly of the $C_{(3)}:C_{(4)}$ double bond, not of part of it, the whole energy of polarographic reduction of the double bond must obviously be taken, i.e. the half-wave potential of the second wave, corresponding to the sum of the electron affinities of the coumarin molecule and of its radical. It is probable that, as in polarography, the transfer of electrons in a spectroscopic experiment takes place in stages (transfer of the first electron followed by that of the second, and so on).

‡This reference appears to be in error (Ed. of Translation).

The polarographic and spectral characteristics found by the Reviewer for naturally occurring coumarins show (Table 5) a deviation of 0.4–0.5 eV from a linear relation only with five compounds (angelicin, acetylumbelliferone, imperatorin, 4-methylumbelliferone, and xanthotoxin): with all the remaining compounds a precise correlation between optical and electrochemical characteristics is observed, which enables half-wave potentials in the coumarin series to be calculated from the energy of the longer-wavelength absorption band.

Table 5. Electrochemical and optical characteristics of naturally occurring coumarins.

Compound	$-E_{1/2}$, V	ϵ, eV	λ_m, nm	$h\nu$, eV
Coumarin	1.97	3.94	309	4.00
6-Methylcoumarin	1.92	3.84	320	3.88
Umbelliferone	1.84	3.68	324	3.83
Acetylumbelliferone	1.80	3.60	310	4.00
Fraxinol	1.93	3.86	308	4.03
Osthole	2.08	4.16	321	3.87
Aesculin	1.74	3.98	333	3.73
Acetylfraxinol	1.88	3.76	312	4.00
Psoralen	1.95	3.90	292	4.25
Angelicin	1.89	3.78	289	4.30
Aesculetin	1.78	3.56	347	3.60
Isopimpinellin	1.81	3.62	308	3.95
Imperatorin	1.85	3.70	299	4.15
Bergapten	1.86	3.72	309	4.00
7-Isopentenyloxycoumarin	2.08	4.16	322	3.86
Sphondin	1.90	3.80	303	4.10
Umbelliprenin	2.05	4.10	309	4.00
Cnidicin	1.86	3.72	308	4.06
Cnidilin	1.91	3.82	308	4.06
Xanthotoxin	1.85	3.70	298	4.16
Xanthotoxol	2.00	4.00	306	4.06
4-Methylumbelliferone	1.74	3.48	315	3.93
Farnesiferol	1.86	3.72	322	3.86

2. Mass-spectral Characteristics and Half-wave Potentials

Electron bombardment of coumarin produces molecular ions[161–163], whose measured appearance potential was corrected for the contact potential of the nickel–molybdenum system (the ion source)[164]. This can be accepted as the ionisation potential, which in the Reviewer's experiments was around 4.0 eV. The correlation of spectral, mass-spectral, and polarographic data enables spectral characteristics to be used to find ionisation potentials, and the equality of the latter to the half-wave potentials as a criterion of the degree of homolysation of the molecule[154, 155] and the consequent possibility of formation of a charge-transfer complex, since the half-wave potentials can be used to find electron affinities[159].

3. Electron Affinity and Half-wave Potential

Since half-wave potentials are often used to estimate electron affinities, it was of interest to compare these quantities. Experimental determination of electron affinity is usually based on the examination of charge-transfer complexes, the theory of which was developed by Mulliken[165], or on polarography[159,166]. The Reviewer has calculated electron affinity A by Scott and Becker's method[167] based on the equation

$$A = -\alpha^2 - \left(m_{\overline{N}} - \frac{\eta+1}{\eta} N\right)\beta^0,$$

obtained from approximate quantum-chemical calculations, in which $\alpha^2 = -9.878$ eV, $\beta^0 = 2.110$ eV, $m_{\overline{N}}^{12}$ is the Hückel coefficient of the highest occupied orbital or of the anion, η is the number of π electrons in the neutral molecule, and N is a constant.

Table 6.

Compound	η	$m_{\overline{N}}$ (Ref.12)	$-A$, eV	$-E_{1/2}$, V
Coumarin	12	1.2391	3.70	1.40
Herniarin	12	1.1903	3.80	1.44
4-Methylumbelliferone	12	1.2000	3.93	1.61
Psoralen	16	1.1200	3.65	1.44

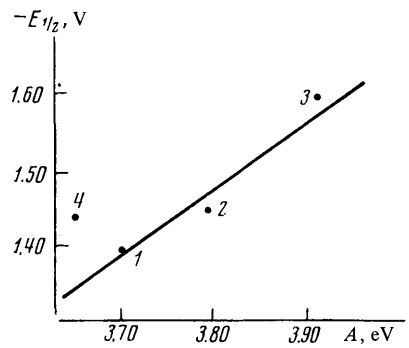


Figure 6. Relation between half-wave potential and electron affinity of coumarins: 1) coumarin; 2) herniarin; 3) 4-methylumbelliferone; 4) psoralen.

The calculated results are given in Table 6. The graphical relation between the two quantities shows (Fig. 6) that the polarographic half-wave potential varies linearly with the electron affinity of the coumarins.

4. Dipole Moments and Half-wave Potentials

Data on the dipole moments of coumarins are extremely limited, but the Reviewer's attempts to calculate them by quantum-chemical methods[15,168,169] gave overestimates.

For most substances the wave is considered[20] to move to less negative potentials with increase in the dipole moment. The present case constitutes an exception to the general rule (Fig. 7), which merits special examination.

5. Nuclear Magnetic Resonance and Half-wave Potential

The fine structure of an organic molecule is characterised by the chemical shift and the spin distribution constant, which is why n.m.r. spectroscopy[170,171] finds such great use for confirming the conformations of molecules, the distribution of electron density on the atoms, found by other physicochemical methods (spectroscopy, dipole moments) and by quantum-chemical methods. Comparison of polarographic and n.m.r. results is of the greatest

interest, since it enables the electrochemically active units of the molecule, i.e. the sites of lowest electron density, to be determined.

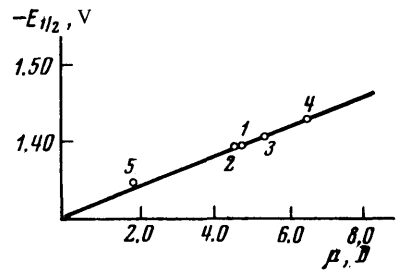


Figure 7. Relation between half-wave potentials and dipole moments (determined according to Debye) for coumarins: 1) coumarin; 2) coumarin-6-sulphonic acid; 3) 6-methylcoumarin; 4) 6-aminocoumarin; 5) 6-nitrocoumarin.

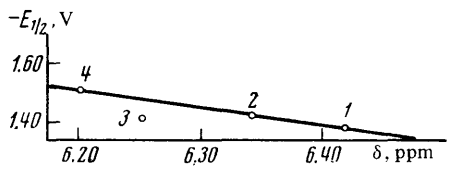


Figure 8. Relation between half-wave potential and chemical shift for coumarins: 1) coumarin; 2) fraxinyl acetate; 3) herniarin; 4) 7-hydroxyisopentenyloxycoumarin.

Nuclear magnetic resonance data for coumarins[12,172-174] show that the $C_{(3)}:C_{(4)}$ double bond is the site of lowest electron density, and the 2-pyrone carbonyl group the site of maximum electron density, in agreement with quantum-chemical calculations[12,131] and the vector diagram of the dipole moment of the coumarin molecule. This is confirmed by the comparison of half-wave potentials and chemical shifts plotted in Fig. 8.

6. Diffusion-current Constants and Molecular Mass

Increase in molecular mass entails a decrease in the diffusion-current constant (Fig. 9). This provided a method for estimating the molecular masses of coumarins, and also for estimating the constant from the latter, which is very important in calculating the number of electrons and interpreting the mechanism of electrode reactions.

V. METHODS OF POLAROGRAPHIC ANALYSIS

The polarographic method of analysis is widely used for coumarins[30,31,35,41-46,48,51,54,57,175] and the related chromones[43,175]. The techniques employed are based on displaying the depolarising ability of these compounds at a dropping mercury electrode in the cathodic potential range. Novel physicochemical properties of coumarins detected in this way are of the greatest interest.

Investigation of the mechanism of the chain autoxidation of sulphites revealed that naturally occurring coumarins have a considerable inhibiting effect[46]. A linear dependence exists between the duration of inhibition and the concentration of the coumarins (within the range 10^{-4}–10^{-6}M), which can be used in quantitative analysis. Different coumarins have different ranges of linearity. The inhibiting effect is detected from the change in the rate of fall of the maximum oxygen content in the presence of a sulphite by polarography with 0.01% potassium chloride solution as supporting electrolyte. The sensitivity of this method of analysis (10^{-7} M) is hundredfold that of methods based on the electrochemical reduction of coumarins (10^{-5} M). It will give good results in the analysis of polarographically inactive coumarins (dihydrocoumarin, 4-hydroxycoumarin derivatives) and of coumarins that have poorly developed polarographic waves (hydroxycoumarins).

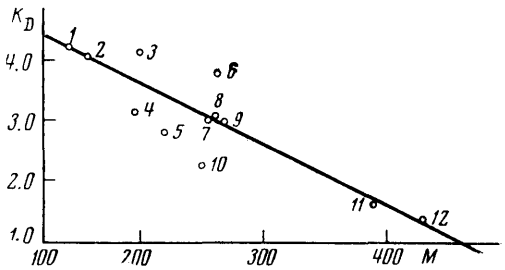


Figure 9. Dependence of K_D on molecular mass for the coumarins: 1) coumarin; 2) 6-methylcoumarin; 3) xanthotoxin; 4) psoralen; 5) isobergapten; 6) 7-isopentenyloxycoumarin; 7) fraxinol; 8) imperatorin; 9) fraxinyl acetate; 10) pimpinellin; 11) farnesiferol; 12) athamantin.

A very urgent problem in the chemistry of coumarins is the determination of molecular mass. Hitherto it has been found via the diffusion coefficient by the use of diffusion currents, which is time-consuming and not always accurate. The molecular mass can be determined experimentally by polarography using a standard compound. This gives a formula, based on the Ilkovič and Stokes–Einstein equations[156], from which diffusion coefficients are absent[47,176]:

$$M_x = \left(\frac{10^{16} I_{st}^4 \cdot M_{st} \cdot c_x^4}{I_x^4 \cdot c_{st}^4} \right)^{1/4},$$

where M_X is the molecular mass of the test coumarin, I_{st} and I_X (in μA) are the limiting currents of standard and test coumarin solutions, M_{st} is the molecular mass of the standard coumarin derivative, c_{st} (mM) is the concentration of the standard coumarin solution, and c_X (%) that of the test solution. The relative error of the determination is ±2.5%. The method developed by the Reviewer is far simpler than the mass-spectral method with respect to equipment and more reliable than Rast's method. The methods of analysis of coumarins are applicable also to other classes of organic compounds, and have entered the USSR State Pharmacopoeia[102].

The use of chromatographic methods—paper, thin-layer, and gel—for the preliminary separation of mixtures of coumarins and chromones extends the possibilities of polarography in the investigation and the analysis of these compounds[98,177-180].

VI. APPLICATION OF POLAROGRAPHY TO THE CHEMISTRY AND BIOLOGY OF COUMARINS

1. Kinetics of Cleavage of the Lactone Ring

As already mentioned, the kinetics of cleavage of the lactone ring is not only of practical interest, from the point of view of finding optimum conditions for the analysis, synthesis, and isolation of coumarins, but also of theoretical interest, since by analogy with benzoic esters the determination of rate constants should permit establishment of a scale of the values of σ required for the correlation of chemical structure with the physicochemical properties of coumarins.

The polarographic method is absolute in recording the lactone form of coumarin in solution, since the cleaved form—hydroxycinnamic acid—is not established in several supporting electrolytes. Other methods (spectrophotometric, conductometric, potentiometric) enable relative measurements to be made[64-66]. An oscillographic technique enables measurements to be made at high rates of fission of the lactone ring.

Table 7. Rate constants (M^{-1} s^{-1}) of cleavage of the lactone ring of coumarin in various media.

Solvent compound	concn., vol.%	LiOH	NaOH	KOH	$(C_2H_5)_4$ NOH
Water	100.0	0.338	0.347	0.388	0.130
Methanol	25.0	—	0.227	—	—
Methanol	30.8	—	0.101	—	—
Methanol	50.0	0.073	0.069	0.082	—
Methanol	70.0	—	0.020	—	—
Ethanol	19.0	—	0.146	—	—
Ethanol	30.8	—	0.131	—	—
Ethanol	50.0	0.132	0.124	0.129	—
Ethanol	70.0	—	0.056	—	—
Propan-1-ol	19.0	—	0.162	—	—
Propan-1-ol	30.8	—	0.075	—	—
Propan-1-ol	50.0	0.032	0.0638	0.054	0.059
Propan-1-ol	70.0	—	0.062	—	—
Propan-1-ol	96.0	—	0.645	—	—
Propan-2-ol	19.0	—	0.266	—	—
Propan-2-ol	30.8	—	0.124	—	—
Propan-2-ol	50.0	0.106	0.124	0.108	0.131
Propan-2-ol	70.0	—	0.116	—	—
Dimethylformamide	50.0	0.035	0.036	0.047	—

Application of polarography[56] and colorimetry[72,182] to isomolar series[181] shows that coumarin reacts in equimolecular proportions with a base. The reaction is of the second order[67,183]. The investigations have been made in aqueous-alcoholic solutions in the presence of various bases—ammonium, lithium, sodium, potassium, and tetraethylammonium hydroxides—and reaction rate constants have been determined for various concentrations of the alcohol (Table 7). The reaction does not take place in purely alcoholic solvents (methanol, ethanol, propan-1(or 2)-ol). The rate constant increases with decrease in the mole fraction of the organic solvent and in the inductive effect of the substituent but with increase in the strength of the base. The more negative the half-wave potential of coumarin, the more difficult is cleavage of the lactone ring. All the researches show that this process depends on many factors and involves water molecules.

2. Complex Formation in the Coumarin Series

Polarography is widely used to investigate complex formation[75,76,184]. The first polarographic study of the formation by aesculetin of complexes with copper, gold, tin, and rhodium salts has been made by the Reviewer. Copper salts give stable complexes, but the process is protracted, taking place in several stages with formation of a mixture of complexes differing in chemical structure, as chromatography[1,185,186] and spectroscopy[187,188] have shown. Earlier spectral results[187] are dubious in view of the close similarity in physicochemical properties of the components of the aggregate complex.

The presence of a complex is established polarographically from the difference between the half-wave potentials of metal and coumarin. The stoichiometric ratio of copper and aesculetin in the complex has been determined[162]. The other complexes—with rhodium, gold, and tin—have only been identified polarographically during their formation.

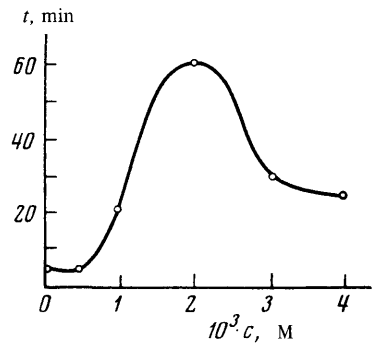


Figure 10. Dependence of the duration t of inhibition by coumarin of the chain autoxidation of sodium sulphite on its concentration c.

3. Inhibition of Chain Reactions

A form of chain reaction is the autoxidation of sulphites[189-193], which can conveniently be investigated by a polarographic method, since the reaction is studied by measuring the concentration of oxygen in the solution. The Reviewer has been the first to use polarography to investigate this reaction. Coumarins exert a considerable inhibiting effect[194] (Fig. 10), which consists in significant retardation of the rate of autoxidation by interaction of the added coumarin molecules with radicals appearing during the reaction. The inhibiting effect becomes stronger with increase in the molecular mass of the coumarin, in the degree of branching of the molecule, and in the number of phenolic groups.

The mechanism of this reaction, involving ion-radical processes, models redox processes occurring in the living organism. Therefore the useful pharmacological action of many medicinal and biologically active compounds, including coumarin derivatives, is accompanied by a harmful inhibiting effect on redox processes in healthy cells of the organism. In the case of pathological processes (cancer) the inhibiting effect may be of positive value: indeed several coumarins are used for the treatment of malignant diseases[195]. Detailed investigation of the mechanism of the inhibiting effect of coumarins (based on mathematical equations and models) will help to reveal the biological functions of coumarins in plant and animal organisms at a higher level, and thereby establish a more exact model of the dependence of pharmacological action on chemical structure.

The formation of hydrogen peroxide during the autoxidation of sulphites has also been shown by a polarographic method—from the increase in height of the second oxygen wave—which has permitted a more thorough investigation of the mechanism of this reaction. On this basis it is possible to study the mechanism of the aging of coumarins, and also to develop methods for their accelerated aging, enabling the useful life of coumarin preparations to be predicted.

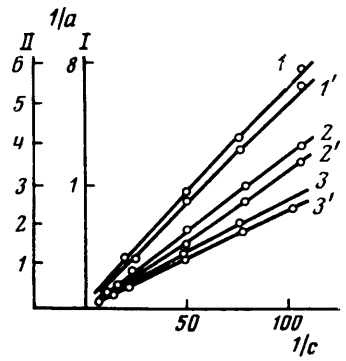


Figure 11. Adsorption isotherms for xanthotoxin on silica gel with: I) column chromatography; II) thin-layer chromatography (primed numerals) and different mobile phases: 1) chloroform; 2) acetone; 3) methanol.

4. Adsorption Phenomena

The investigation of adsorption phenomena at the mercury–solution interface is one of the most important fields in the electrochemistry and the physical chemistry of surface phenomena, permitting a more profound and detailed interpretation of polarographic investigations. The occurrence of adsorption phenomena in solutions of coumarins has been established[17,32] from the character of the electrocapillary curves and the Gibbs adsorption isotherms[118,119] plotted from data obtained by means of a dropping mercury electrode. Coumarin is desorbed on the negative branch of the electrocapillary curve. Adsorption phenomena supplement certain information on electrochemical reactivity, and also model exchange processes at biological membranes. In combination with column chromatography, furthermore, the polarographic method enables adsorption isotherms (Fig. 11) to be obtained for coumarins[196,197].

A new method of plotting the adsorption isotherms of organic compounds[53], based on thin-layer chromatography, involves measurement of the difference between the distances advanced by the solvent front and by the substance Δlf.

5. Solubility of Coumarins

The great sensitivity of the polarographic method and the possibility of electrochemical reduction in the most diverse media enable it to be widely used to determine the solubility of coumarins by polarographing saturated solutions after dilution with the appropriate solvent.

Table 8. Solubility of coumarin in various solvents at 20°C (c is the content of the substance in grams per 100 ml solution).

Solvent	c	Solvent	c
Methanol	15.10	Butanol	6.35
Ethanol	8.30	Pentanol	4.10
Propan-1-ol	5.80	Dioxan	39.40
Propan-2-ol	7.20	Acetone	44.40

At the same time the solubility was determined refractometrically[41,198]. Table 7 shows that the solubility increases with the number of carbon atoms in the alcohol molecule.

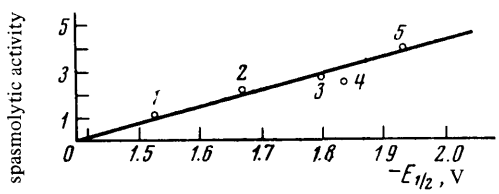


Figure 12. Dependence of spasmolytic action of coumarins (according to results obtained in the Pharmacology Laboratory of the Kharkov Chemical and Pharmaceutical Scientific Research Institute) on the half-wave potential: 1) coumarin; 2) umbelliferone; 3) 4-methylumbelliferone; 5) aesculin; 6) aesculetin.

6. Half-wave Potential and Biological Activity

The biological activity of organic molecules can be assessed from a combination of pharmacological, biochemical, and physicochemical investigations. Considerable progress can be made in this field by the application of polarography according to the following scheme: (i) comparative estimation, based on the half-wave potentials,

of oxidation and reduction, ionisation potentials, and electron affinities, parametrising the principal physicochemical and biological properties of organic molecules; (ii) comparative assessment of the quantum-chemical parameters and the degrees of homolysation of organic molecules by comparing the polarographic half-wave potentials with quantities—energy of longer-wavelength absorption band, refraction, dipole moments—obtained by other physicochemical methods; (iii) experimental determination of inhibiting effect on chemical models and enzyme systems from the duration of inhibition found from the fall in wave height; (iv) determination of metabolic products; (v) comparative study of chemical and electrochemical stability of pharmacologically important units in the molecules; and (vi) determination of resorptive adsorption capacity.

We have compared the half-wave potentials with the spasmolytic activities of naturally occurring coumarins (Fig. 12) from Khadzhai's results[199]. The greater the electron density of the 2-pyrone ring in the coumarin system the greater the spasmolytic activity (e.g. the activity of simple coumarins is far lower than that of furocoumarins, which applies also to the half-wave potentials).

A polarographic method has been used also to study the photo-oxidation of furocoumarins[200] in the living organism from the consumption of oxygen.

REFERENCES

1. G.A.Kuznetsova, "Prirodnye Kumariny i Furokumariny" (Naturally Occurring Coumarins and Furocoumarins), Nauka, Leningrad, 1967.
2. M.G.Pimenov, "Perechen' Rastenii–Istochnikov Kumarinovykh Soedinenii" (List of Plant Sources of Coumarin Compounds), Nauka, Leningrad, 1971.
3. J.B.Harborne (Editor), "Biochemistry of Phenolic Compounds" (Translated into Russian), Mir, Moscow, 1968.
4. T.O.Soine, J.Pharm.Sci., 53, 231 (1964).
5. "Spravochnik Khimika" (Chemist's Handbook), Khimiya, Leningrad, 1965, Vol. 4, p. 381.
6. K.Venkataraman (Editor), "The Chemistry of Synthetic Dyes" (Translated into Russian), Khimiya, Leningrad, 1974, Vol. 3, p. 1711.
7. M.S.Dinaburg, "Svetochuvstvitel'nye Soedineniya" (Light-sensitive Compounds), Khimiya, Leningrad, 1964, p. 22.
8. Yu.P.Yampol'skii, "Izluchenie Lazera i Khimicheskaya Reaktsiya" (Laser Emission and Chemical Reaction), Znanie, Moscow, 1973, p. 22.
9. A.A.Shamshurin and M.Z.Krimer, "Fiziko-khimicheskie Svoistva Organicheskikh Yadokhimikatov i Regulyatorov Rosta, Spravochnik" (Handbook of the Physicochemical Properties of Organic Chemical Poisons and Growth Regulators), Nauka, Moscow, 1966, p. 84.
10. A.I.Ban'kovskii, "Lekarstvennye Rasteniya" (Medicinal Plants), Kolos, Moscow, 1969, Vol. 15, p. 3.
11. M.E.Perel'son, Aptechnoe Delo, 70 (1963).
12. M.E.Perel'son, in "Lekarstvennye Rasteniya" (Medicinal Plants), Kolos, Moscow, 1969, p. 25.
13. I.P.Kovalev and E.V.Titov, "Infrakrasnye Spektry Pogloshcheniya Nekotorykh Grupp Prirodnykh Soedinenii (Atlas Spektrov)" (Infrared Absorption Spectra of Some Groups of Naturally Occurring Compounds—Atlas of Spectra), Izd. Khar'kovsk. Gos. Univ. Gor'kogo, Kharkov, 1966.
14. Yu.E.Orlov and A.P.Prokopenko, Khim. Prirod. Soed., 216 (1969).
15. V.I.Minkin, O.A.Osipov, and Yu.A.Zhdanov, "Dipol'nye Momenty v Organicheskoi Khimii" (Dipole Moments in Organic Chemistry), Khimiya, Leningrad, 1968.
16. "Spravochnik po Dipol'nym Momentam" (Handbook of Dipole Moments), Vysshaya Shkola, Moscow, 1971.
17. V.S.Griffiths and J.B.Westmore, J.Chem.Soc., 4941 (1963).
18. S.K.K.Jatkar and C.M.Deshpande, J.Indian Chem. Soc., 37, 19 (1960).
19. S.V.Anatakrishnan and J.Jacobi, J.Indian Chem. Soc., 7, 234 (1969) [sic].
20. S.A.Voitkevich, Zhur. Fiz. Khim., 26, 869 (1952).
21. N.G.Bakhshiev, M.I.Knyazhanskii, V.I.Minkin, O.A.Osipov, and G.V.Saidov, Uspekhi Khim., 38, 1644 (1968) [Russ. Chem. Rev., No. 9 (1968)].
22. I.P.Beletskaya (Editress), "Novye Problemy Fizicheskoi Organicheskoi Khimii" (New Problems of Physical Organic Chemistry) (Translations into Russian), Mir, Moscow, 1969.
23. Ya.P.Stradyn', V.P.Kadysh, and S.A.Giller, Khim. Geterotsikl. Soed., 149 (1974).
24. A.P.Terent'ev and L.A.Yanovskaya, in "Reaktsii i Metody Issledovaniya Organicheskikh Soedinenii" (Reactions and Methods of Investigation of Organic Compounds), Goskhimizdat, Moscow, 1967, p. 5.
25. J.Volke, in "Physical Methods in Heterocyclic Chemistry" (edited by A.R.Katritzky) (Translated into Russian), Khimiya, Moscow, 1966, p. 227.
26. A.Banchetti and S.Silvesri, Farmaco. Ed. Pr., 25, 186 (1970).
27. O.Čapka, Coll. Czech. Chem. Comm., 15, 965 (1950).
28. E.Knobloch, Coll. Czech. Chem. Comm., 25, 3330 (1960).
29. E.Knobloch, Adv. Polarography, Coll. Czech. Chem. Comm., 2, 875 (1960).
30. Y.Mashiko, J.Pharm.Soc.Japan, 72, 18 (1952).
31. M.Skora-Zietek, Farm. polska, 22, 254 (1966).
32. V.S.Griffiths and J.B.Westmore, J.Chem.Soc., 1704 (1962).
33. A.Foffani, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 14, 281 (1953).
34. A.Foffani, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 14, 418 (1953).
35. M.Brezina and P.Zuman, "Die Polarographie in der Medizin, Biohemie und Pharmazie", Akad. Verlag, Leipzig, 1956, p. 1280.
36. R.Patzak and L.Neugebauer, Monatsh., 82, 662 (1951); 83, 776 (1952).
37. M.Deželic and M.Trkovnik, Croat. Chem. Acta, 33, 209 (1961).
38. M.Deželic and M.Trkovnik, Croat. Chem. Acta, 35, 43 (1963).
39. A.J.Harle and L.E.Lyons, J.Chem.Soc., 1575 (1950).
40. Yu.E.Orlov and A.P.Prokopenko, Zhur. Obshch. Khim., 40, 1159 (1970).
41. Yu.E.Orlov, R.N.Dorofeeva, N.V.Krupskaya, A.P.Prokopenko, and R.V.Piskareva, "II Simpozium po Izucheniyu Prirodnykh Kumarinov, Tezisy Dokladov" (Second Symposium on the Study of Natural Coumarins—Abstracts of Papers), Leningrad, 1970, p. 18.

42. Yu.E.Orlov, "Materialy Vtorogo S'ezda Farmatsevtov" (Proceedings of the Second Congress of Pharmacists), Riga, 1974, p.137.
43. Yu.E.Orlov, N.P.Dzyuba, L.Ya.Sirenko, and R.N.Dorofeeva, Herba Polonica, 17, 72 (1971).
44. Yu.E.Orlov, "Trudy Vseukrainskogo S'ezda Farmatsevtov" (Transactions of the All-Ukrainian Congress of Pharmacists), Lvov, 1972, p.803.
45. Yu.E.Orlov and L.Ya.Sirenko, "Polyarografiya i pH-metriya v Kontrole Khimicheskikh Proizvodstv" (Polarography and pH Measurement in the Control of Chemical Works), Prom.Khim.Reaktivov Osobo Chist.Veshchestv, 18, 173 (1968).
46. Yu.E.Orlov, "Tezisy Dokladov I Nauchnoi Konferentsii Farmatsevtov Urala i Sibiri.K Voprosu Sinteza, Analiza i Stroeniya Nekotorykh Farmakologicheski Aktivnykh Veshchestv" (Synthesis, Analysis, and Structure of Some Pharmacologically Active Substances—Abstracts of Papers at the First Scientific Conference of Pharmacists of the Ural and Siberia), Tyumen, 1973, p.113.
47. Yu.E.Orlov, O.P.Lichino, and O.P.Usol'tseva, "Tezisy Dokladov I Nauchnoi Konferentsii Farmatsevtov Urala i Sibiri. K Voprosu Sinteza, Analiza i Stroeniya Nekotorykh Farmakologicheski Aktivnykh Veshchestv" (Synthesis, Analysis, and Structure of Some Pharmacologically Active Substances—Abstracts of Papers at the First Scientific Conference of Pharmacists of the Ural and Siberia), Tyumen, 1973, p.214.
48. Yu.E.Orlov, O.P.Lichino, and O.P.Usol'tseva, "Tezisy Dokladov I Nauchnoi Konferentsii Farmatsevtov Urala i Sibiri. K Voprosu Sinteza, Analiza i Stroeniya Nekotorykh Farmakologicheski Aktivnykh Veshchestv" (Synthesis, Analysis, and Structure of Some Pharmacologically Active Substances—Abstracts of Papers at the First Scientific Conference of Pharmacists of the Ural and Siberia), Tyumen, 1973, p.75.
49. Yu.E.Orlov, International Symposium on the Nature, Products, and Phytochemistry of the Balkan Flora, Varna, Bulgaria, A, 1971, p.29.
50. Yu.E.Orlov and R.N.Dorofeeva, "Tezisy Dokladov II Vsesoyuznoi Nauchno-tekhnicheskoi Konferentsii po Analiticheskoi Khimii Nevodnykh Rastvorov i ikh Fiziko-khimicheskim Svoistvam" (Abstracts of Papers at the Second All-Union Scientific and Technical Conference on the Analytical Chemistry of Non-aqueous Solutions and their Physicochemical Properties), Kharkov, 1968, p.95.
51. Yu.E.Orlov, N.P.Dzyuba, L.Ya.Sirenko, N.E.Vorob'ev, and N.Ya.Tsarenko, "Novye Issledovaniya v Polyarografii" (New Researches in Polarography), Shtiintsa, Kishinev, 1972, p.110.
52. Yu.E.Orlov and R.V.Piskareva, "Novye Issledovaniya v Polyarografii" (New Researches in Polarography), Shtiintsa, Kishinev, 1972, p.300.
53. Yu.E.Orlov and A.I.Udalov, "Novye Issledovaniya v Polyarografii" (New Researches in Polarography), Shtiintsa, Kishinev, 1972, p.319.
54. Yu.E.Orlov, "Tezisy Dokladov na Otraslevom Nauchno-tekhnicheskogo Soveshchaniya po Primeneniyu Fiziko-khimicheskikh Metodov Analiza v Khimicheskoi Promyshlennosti" (Abstracts of Papers at an Industrial, Scientific, and Technical Conference on the Application of Physicochemical Methods of Analysis in the Chemical Industry), Kharkov, 1966, p.75.
55. Yu.E.Orlov and L.Ya.Sirenko, "Tezisy Dokladov na Otraslevom Nauchno-tekhnicheskogo Soveshchaniya po Primeneniyu Fiziko-khimicheskikh Metodov Analiza v Khimicheskoi Promyshlennosti" (Abstracts of Papers at an Industrial, Scientific, and Technical Conference on the Application of Physicochemical Methods of Analysis in the Chemical Industry), Kharkov, 1966, p.78.
56. Yu.E.Orlov and R.V.Piskareva, Zhur.Obshch. Khim., 45, 2062 (1975).
57. Yu.E.Orlov and T.E.Gulimova, "Vsesoyuznoe Soveshchanie po Analiticheskomu Kontrolyu Proizvodstva Lekarstvennykh i Farmatsevticheskikh Preparatov, Tezisy Dokladov" (All-Union Conference on the Analytical Control of the Production of Medicinal and Pharmaceutical Preparations—Abstracts of Papers), Perm, 1974, p.182.
58. P.Zuman, Chem.listy, 48, 94 (1954).
59. O.Čapka, Proc.Internat.Polarogr.Congr., Prague, 1951, Vol.1, p.699; Vol.3, p.509.
60. J.Kovač, Chem.Zvesti, 8, 272 (1954).
61. S.G.Mairanovskii, A.A.Fainzil'berg, S.S.Novikov, V.A.Novikov, and V.A.Klimova, Dokl.Akad.Nauk SSSR, 125, 351 (1959).
62. H.Böhme and T.Severin, Arch.Pharm., 285, 290, 405, 448, 486 (1957).
63. E.Cingolani, Gazzetta, 89, 985 (1959).
64. B.C.Lippold and E.K.Carret, J.Pharm.Sci., 60, 1019 (1971).
65. K.Bowden, M.J.Nanson, and J.R.Taylor, J. Chem.Soc.(B), 174 (1968).
66. E.K.Carret, B.C.Lippold, and G.B.Milck, J. Pharm.Sci., 60, 396 (1971).
67. C.K.Ingold, "Structure and Mechanism in Organic Chemistry" (Translated into Russian), Mir, Moscow, 1973, p.936.
68. R.C.Elderfield (Editor), "Heterocyclic Compounds" (Translated into Russian), 1954, Vol.2, p.134.
69. G.Giacometti, Atti Accad.naz.Lincei,Rend.Classe Sci.fis.mat.nat., 17, 379 (1954).
70. S.Milstien and G.A.Cohen, J.Amer.Chem.Soc., 94, 9158 (1972).
71. G.K.Nikonov, Aptechnoe Delo, 61 (1964).
72. O.P.Prokopenko and O.O.Tarasenko, Farm.Zhur., 18 (1962).
73. A.P.Prokopenko and D.G.Kolesnikov, "Tezisy Dokladov Konferentsii po Izucheniyu i Ispol'zovaniyu Lekarstvennykh Rastel'nykh Resursov" (Abstracts of Papers at a Conference on the Study and Use of Medicinal Plant Resources), Baku, 1961, p.66.
74. V.D.Bezuglyi, "Polyarografiya v Khimii i Tekhnologii Polimerov" (Polarography in Polymer Chemistry and Technology), Khimiya, Leningrad, 1968.
75. J.Heyrovský and J.Kuta, "Principles of Polarography" (Translated into Russian), Mir, Moscow, 1865.
76. T.A.Kryukova, S.I.Sinyakova, and T.V.Aref'eva, "Polyarograficheskii Analiz" (Polarographic Analysis), Goskhimizdat, Moscow, 1959.
77. S.G.Mairanovskii, "Kataliticheskie i Kineticheskie Volny v Polyarografii" (Catalytic and Kinetic Waves in Polarography), Nauka, Moscow, 1966.
78. A.L.Markman and E.V.Zinkova, Zhur.Obshch. Khim., 27, 1438 (1957).
79. O.H.Wheeler and C.B.Covarrubias, J.Org.Chem., 28, 2015 (1963).

80. M.J.D.Brand and B. Fleet, J.Electroanalyt.Chem., 16, 341 (1968).
81. V.A.Pal'm, "Vvedenie v Teoreticheskuyu Organicheskuyu Khimiyu" (Introduction to Theoretical Organic Chemistry), Vysshaya Shkola, Moscow, 1974.
82. G.Semerano and A.Chisini, Gazzetta, 66, 510 (1936).
83. S.Ono and T.Hayashi, Bull.Chem.Soc.Japan, 26, 268 (1953).
84. A.L.Markman, Uzbek.Khim.Zhur., 50 (1959).
85. L.Schwaer, Coll.Czech.Chem.Comm., 7, 326 (1935).
86. S.Wawzonek, S.C.Wang, and P.Layons, J.Org. Chem., 15, 593 (1950).
87. S.Ono and M.Uehara, Bull.Univ.Osaka Prefect.Ser. A, 5, 139 (1957).
88. J.Seveik and J.Mollin, Acta Univ.Palackianac Olomuc., Fac.Rerum Nat., 9, 231 (1962).
89. M.E.Perel'son, A.V.Tutkevich, Yu.I.Sheinker, and N.P.Gambaryan, Teor.Eksper.Khim., 574 (1966).
90. A.I.Tolmachev and L.M.Shulezhko, Zhur.Obshch. Khim., 37, 387 (1967).
91. A.Hantzsch, Ber., 52, 1539 (1919).
92. T.I.Temnikova, "Kurs Teoreticheskikh Osnov Organicheskoi Khimii" (Course in the Theoretical Principles of Organic Chemistry), Khimiya, Leningrad, 1968, p.231.
93. F.M.Dean, "Naturally Occurring Oxygen Ring Compounds", London, 1963.
94. K.Fries and G.Fickewirth, Annalen, 30, 362 (1908).
95. L.J.Smith and R.O.Deynes, J.Amer.Chem.Soc., 58, 704 (1936).
96. K.G.Ashurst, Trans.Inst.Metal Finishing, 40, 77 (1936).
97. S.G.Mairanovskii and V.N.Pavlov, Izv.Akad. Nauk SSSR, Ser.Khim., 1669 (1967).
98. Yu.E.Orlov, T.E.Gulimova, A.I.Udalov, and G.I.Yadykin, "Materialy III Vserossiiskogo S'ezda Farmatsevtov" (Proceedings of the Third All-Russian Congress of Pharmacists), Sverdlovsk, 1975, p.198.
99. J.Tirouflet and A.Corvaisier, Bull.Soc.chim. France, 535 (1962).
100. P.Zuman and J.Michl, Nature, 192, 655 (1961).
101. V.F.Lavrushin, V.D.Bezuglyi, and G.G.Belous, Zhur.Obshch.Khim., 33, 1667 (1963).
102. "Gosudarstvennaya Farmakopeya SSSR" (USSR State Pharmacopoeia), Meditsina, Moscow, 1968.
103. H.Adkins and F.W.Cox, J.Amer.Chem.Soc., 60, 1151 (1938).
104. D.W.Engelkemeir, T.A.Geissman, W.R.Crowell, and S.L.Friess, J.Amer.Chem.Soc., 69, 155 (1947).
105. T.A.Geissman and S.L.Friess, J.Amer.Chem. Soc., 71, 3893 (1949).
106. J.Volke and V.Szabo, Coll.Czech.Chem.Comm., 23, 221 (1958).
107. O.Čapka and J.Opavsky, Coll.Czech.Chem.Comm., 15, 433 (1950).
108. J.Berka and O.Nesvadba, Čas.čes lekarn., 63, 33 (1950).
109. E.H.Hinreiner, Ph.D.Thesis, University of California, Los Angeles, 1950.
110. S.L.Friess, Proc.Roy.Soc.A, 19, 92 (1944) [sic].
111. S.D.Bailey, P.A.Geary, and A.E.Wald, J.Amer. Pharm.Assoc.Sci.Ed., 40, 280 (1951).
112. W.C.Ellenbogen, E.S.Rump, P.A.Geary, and M.Burke, J.Amer.Pharm.Assoc.Sci.Ed., 40, 287 (1951).
113. Yu.E.Orlov, Candidate's Thesis, Kharkov, 1969.
114. O.Capka, F.Santavý, and J.Malinský, Coll.Czech. Chem.Comm., 15, 953 (1950).
115. N.E.Vorob'ev and N.P.Dzyuba, Farm.Zhur., 19, 18 (1964).
116. N.E.Vorob'ev and N.P.Dzyuba, "IX Mendeleevskii S'ezd po Obshchei i Prikladnoi Khimii. Sektsiya Analiticheskoi Khimii" (Ninth Mendeleev Congress on General and Applied Chemistry—Analytical Chemistry Section), Nauka, 1965, p.78.
117. N.E.Vorob'ev, Candidate's Thesis, Kharkov, 1972.
118. L.D.Partridge, A.C.Tansley, and A.S.Porter, Electrochim.Acta, 11, 517 (1966).
119. G.T.Rogers and K.J.Taylor, Electrochim.Acta, 11, 1685 (1966).
120. B.B.Damaskin, O.A.Petrii, and V.V.Batrakov, "Adsorbtsiya Organicheskikh Soedinenii na Elektrodakh" (Adsorption of Organic Compounds on Electrodes), Nauka, Moscow, 1968.
121. S.I.Zhdanov, Uspekhi Khim., 37, 1390 (1968) [Russ.Chem.Rev., No.8 (1968)].
122. G.J.Hoijtink and J.Van Schooten, Rec.Trav.chim., 71, 1089 (1952).
123. A.Pullman, B.Pullman, and G.Berthier, Bull.Soc. chim.France, 591 (1950).
124. A.Weissberger (Editor), "Elucidation of Structures by Physical and Chemical Methods" (Translated into Russian), Moscow, 1967, Vol.1, p.453.
125. V.A.Pal'm, "Osnovy Kolichestvennoi Teorii Organicheskikh Reaktsii" (Principles of a Quantitative Theory of Organic Reactions), Khimiya, Leningrad, 1967.
126. Yu.A.Zhdanov and V.I.Minkin, "Korrelyatsionnyi Analiz v Organicheskoi Khimii" (Correlation Analysis in Organic Chemistry), Izd.Rostovsk.Univ., 1966.
127. P.Zuman, Coll.Czech.Chem.Comm., 25,3225 (1960).
128. P.Zuman, Coll.Czech.Chem.Comm., 27, 2035 (1962).
129. V.A.Pal'm, Uspekhi Khim., 30, 1069 (1961) [Russ. Chem.Rev., No.9 (1961)].
130. A.Streitwieser, "Molecular Orbital Theory for Organic Chemists" (Translated into Russian), Mir, Moscow, 1965.
131. B.Pullman and A.Pullman, "Quantum Biochemistry" (Translated into Russian), Mir, Moscow, 1965.
132. A.E.Lutskii, Teor.Eksper.Khim., 753 (1968).
133. Ya.P.Stradyn' and E.S.Levin, "Uspekhi Elektrokhimii Organicheskikh Soedinenii" (Advances in the Electrochemistry of Organic Compounds), Nauka, Moscow, 1966, p.82.
134. L.G.Feoktistov, "Uspekhi Elektrokhimii Organicheskikh Soedinenii" (Advances in the Electrochemistry of Organic Compounds), Nauka, Moscow, 1966, p.135.
135. S.I.Zhdanov, "Uspekhi Elektrokhimii Organicheskikh Soedinenii" (Advances in the Electrochemistry of Organic Compounds), Nauka, Moscow, 1966, p.144.
136. Yu.P.Kitaev and T.V.Troepol'skaya, "Progress Elektrokhimii Organicheskikh Soedinenii" (Progress in the Electrochemistry of Organic Compounds), Nauka, Moscow, 1969, Vol.1, p.176.

137. N.P.Kir'yanov and E.D.Movgan, Khim.Prirod. Soed., 383 (1966).
138. P.A.Zhukov, A.P.Prokopenko, and D.G.Kolesnikov, Med.Prom.SSSR, 32 (1965).
139. R.W.Taft, Jnr., "Separation of Polar, Steric, and Resonance Effects in Reactivity", Chapter in "Steric Effects in Organic Chemistry" (Edited by M.S.Newman) (Translated into Russian), Inostr. Lit., Moscow, 1960.
140. L.P.Hammett, "Fundamentals of Physical Organic Chemistry" (Translated into Russian), Mir, Moscow, 1972.
141. P.Zuman, Adv.Polarography, 3, 812 (1960).
142. O.K.Davtyan, "Kvantovaya Khimiya" (Quantum Chemistry), Vysshaya Shkola, Moscow, 1962.
143. K.Higasi, H.Baba, and A.Rembaum, "Quantum Organic Chemistry" (Translated into Russian), Mir, Moscow, 1967.
144. H.N.Jaffé, Chem.Rev., 53, 191 (1953).
145. D.H.McDaniel and H.C.Brown, J.Org.Chem., 23, 420 (1958).
146. L.I.Lagutskaya and V.A.Dadali, Reaktsionnaya Sposobnost' Org.Soed., 4, 583 (1967).
147. N.P.Shimanskaya and V.D.Bezuglyi, "Stsintillyatory i Stsintillyatsionnye Materialy" (Scintillators and Scintillation Materials), Izd.Khar'kovsk.Univ., 1963, p.63.
148. S.Marcus and S.Miller, J.Phys.Chem., 68, 331 (1964).
149. C.Bannett and P.J.Elving, Coll.Czech.Chem. Comm., 25, 3213 (1960).
150. M.Rapoport, C.Hancock, and E.Meyers, J.Amer. Chem.Soc., 83, 3489 (1961).
151. N.Deno and A.Sehriesheim, J.Amer.Chem.Soc., 77, 3051 (1955).
152. J.Odata and J.Tacogi, J.Amer.Chem.Soc., 80, 3591 (1958).
153. A.Maccoll, Nature, 163, 178 (1949).
154. L.P.Zalukaev, "Gomolizatsiya Organicheskikh Molekul" (Homolysation of Organic Molecules), Izd.Voronezhsk.Univ., Voronezh, 1968.
155. L.P.Zalukaev, "Obmennye Vzaimodeistviya v Organicheskoi Khimii" (Exchange Reactions in Organic Chemistry), Izd.Voronezhsk.Univ., Voronezh, 1974.
156. E.A.Moelwyn-Hughes, "Physical Chemistry" (Translated into Russian), Vol.1, Inostr.Lit., Moscow, 1962. "Physical Chemistry" (Translated into Russian), Vol.2, Inostr.Lit., Moscow, 1962.
157. Z.R.Grabovskii, Zhur.Fiz.Khim., 33, 728 (1959).
158. E.N.Gur'yanova, I.P.Gol'dshtein, and I.P.Romm, "Donorno-aktseptornaya Svyaz'" (The Donor–Acceptor Bond), Khimiya, Moscow, 1973.
159. V.N.Kondrat'ev (Editor), "Energiya Razryva Khimicheskikh Svyazei, Potentsialy i Srodstvo k Elektronu" (Energy of Rupture of Chemical Bonds, Ionisation Potentials, and Electron Affinity), Nauka, Moscow, 1974.
160. O.V.Sverdlova, "Elektronnye Spektry v Organicheskoi Khimii" (Electronic Spectra in Organic Chemistry), Khimiya, Leningrad, 1973.
161. C.S.Barnes and J.L.Occolowitz, Austral.J.Chem., 17, 975 (1964).
162. N.S.Vul'fson, V.I.Zaretskii, and V.G.Zaikin, Izv. Akad.Nauk SSSR, Ser.Khim., 1104 (1964).
163. N.S.Vul'fson and L.S.Golovkina, Uspekhi Khim., 44, 1306 (1975) [Russ.Chem.Rev., No.7 (1975)].
164. V.S.Fomenko, "Emissionnye Svoistva Elementov i Khimicheskikh Soedinenii" (Emission Properties of Elements and Chemical Compounds), Kiev, 1964.
165. R.S.Mulliken, J.Amer.Chem.Soc., 74, 811 (1952).
166. F.Gutman and L.E.Lyons, "Organic Semiconductors" (Translated into Russian), Mir, Moscow, 1970.
167. D.R.Scott and R.S.Becker, J.Phys.Chem., 66, 2713 (1962).
168. R.Dandel, R.Lefebvre, and C.Moser, "Analytical Chemistry", Interscience, New York, 1961.
169. A.Veillard and B.Pullman, Compt.rend., 253, 2418 (1961).
170. J.W.Emsley, J.Feeney, and L.H.Sutcliffe, "High-resolution Nuclear Magnetic Resonance Spectroscopy" (Translated into Russian), Mir, Moscow, 1968.
171. L.A.Kazitsina and N.B.Kupletskaya, "Primenenie UF-, IK-, i YaMR-spektroskopii v Organicheskoi Khimii" (Application of Ultraviolet, Infrared, and Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry), Vysshaya Shkola, Moscow, 1971.
172. M.E.Perel'son, Yu.N.Sheinker, G.P.Syrova, and Kh.F.Turgin, Khim.Prirod.Soed., 6 (1970).
173. S.S.Dahrmatti, G.Govil, C.R.Kanekar, C.L.Khetrapal, and J.P.Virmani, Proc.Indian Acad.Sci., A56, 71 (1962).
174. Yu.N.Sheinker, G.Yu.Pek, and M.E.Perel'son, Dokl.Akad.Nauk SSSR, 158, 1382 (1964).
175. Yu.E.Orlov and N.P.Dzyuba, Med.Prom.SSSR, 19, 44 (1965).
176. Yu E.Orlov and O.P.Lichino, Khim.Prirod.Soed., 657 (1974).
177. Yu.E.Orlov and N.P.Dzyuba, "Molekulyarnaya Khromatografiya" (Molecular Chromatography), Nauka, Moscow, 1964, p.158.
178. Yu.E.Orlov and N.P.Dzyuba, Farm.Zhur., 20, 36 (1965).
179. Yu.E.Orlov, "Simpozium Vsesoyuznogo Nauchnogo Farmatsevticheskogo Obshchestva 'Sintez i Analiz', Tezisy Dokladov" (Symposium of the All-Union Pharmaceutical Science Society "Synthesis and Analysis"—Abstracts of Papers), Lvov, 1966, p.201.
180. Yu.E.Orlov, N.P.Dzyuba, and R.N.Dorofeeva, "Vsesoyuznyi Simpozium po Metodam Analiza Lekarstvennykh Sredstv, Tezisy Dokladov" (All-Union Symposium on Methods of Analysis of Medicinals—Abstracts of Papers), Riga, 1969, p.45.
181. V.M.Peshkova and M.I.Gromova, "Prakticheskoe Rukovodstvo po Spektrofotometrii" (Practical Manual of Spectrophotometry), Izd.Moskovsk.Univ., 1965.
182. B.Kakač and J.Vejedelek, "Handbuch der Kolorimetrie, Kolorimetrie in der Pharmazie", Jena, 1962, Vol.1, p.624.
183. A.I.Brodskii, "Fizicheskaya Khimiya" (Physical Chemistry), Goskhimizdat, 1948.
184. F.J.C.Rossotti and H.Rossotti, "The Determination of Stability Constants" (Translated into Russian), Mir, Moscow, 1965.
185. E.Stahl (Editor), "Thin-layer Chromatography" (Translated into Russian), Mir, Moscow, 1965.
186. I.Hais and H.Macek (Editors), "Paper Chromatography" (Translated into Russian), Inostr.Lit., Moscow, 1962.
187. M.A.Finkel'shteinante, G.I.Stretskite, and Zh.V.Panina, Nauch.Trudy Vuzov Litovsk.SSR, Khim.Tekhnol., 15 (1973).

188. M. I. Bulatov and I. P. Kalinkin, "Prakticheskoe Rukovodstvo po Fotokolorimetricheskim i Spektrofotometricheskim Metodam Analiza" (Practical Manual of Photocolorimetric and Spectrophotometric Methods of Analysis), Leningrad, 1968.
189. N. N. Semenov, "Tsepnye Reaktsii" (Chain Reactions), Goskhimtekhizdat, 1934, p. 458.
190. H. L. J. Bäkstrom, J. Amer. Chem. Soc., **49**, 1460 (1927).
191. H. L. J. Bäkstrom and H. M. Alyea, J. Amer. Chem. Soc., **51**, 90 (1929).
192. H. M. Alyea, J. Amer. Chem. Soc., **55**, 575 (1933).
193. F. Haber, Naturwiss., 19, 450 (1931).
194. T. S. Tsurkan, L. A. Kiseleva, and Yu. E. Orlov, "Nauchno-prakticheskaya Konferentsiya Farmatsevtov Kurskoi Oblasti, Programmnye Materialy" (Scientific and Practical Conference of Pharmacists of the Kursk Region—Programme Documents), Kursk, 1972, p. 148.
195. G. A. Kuznetsova, "Terpenoidy i Kumariny" (Terpenoids and Coumarins), Nauka, Moscow and Leningrad, 1965.
196. B. V. Aivazov, "Prakticheskoe Rukovodstvo po Khromatografii" (Practical Manual of Chromatography), Vysshaya Shkola, Moscow, 1968.
197. A. A. Zhukhovitskii (Editor), "Rukovodstvo po Gazovoi Khromatografii" (Manual of Gas Chromatography—Translations into Russian), Mir, Moscow, 1969.
198. B. V. Ioffe, "Refraktometricheskie Metody Khimii" (Refractometric Methods in Chemistry), Khimiya, Leningrad, 1971.
199. Ya. I. Khadzhai, "Terpenoidy i Kumariny" (Terpenoids and Coumarins), Nauka, Moscow and Leningrad, 1965.
200. E. Fornasari and G. Rodighiero, Adv. Polarography, **2**, 1093 (1960).

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