Other condensation products were obtained by a similar method.

LITERATURE CITED

1. E. P. Opanasenko, V. I. Skalkina, P. V. Prisyazhnyuk, et al., Khim. Farm. Zh., 6, 8 (1972).
2. G. T. Pilyugin, P. V. Prisyazhnyuk, G. K. Palii, et al., Khim. Farm. Zh., 7, 21 (1975).
3. G. T. Pilyugin, N. P. Perepechko, N. I. Verteletskaya, et al., Inventor's Certificate No. 412870; Otkrytiya, 4, 8 (1974).
4. B. P. Lugovkin, Zh. Obshch. Khim., 34, 3107 (1964).
5. V. I. Slesarev, B. A. Ivin, N. A. Smorygo, et al., Zh. Org. Khim., 6, 1313 (1970).
6. B. M. Gutsulyak, Usp. Khim., 41, 346 (1975).
7. A. M. Khaletskii, Pharmaceutical Chemistry [in Russian], Leningrad (1966), pp. 382-405.
8. O. Döebner and W. Miller, Ber. Dtsch. Chem. Ges., 16, 2464 (1883).
9. B. I. Ardashev, Zh. Obshch. Khim., 19, 550 (1949).
10. Z. Ya. Krainer, "Synthesis, structure, and color of quinoindothiocarbocyanins," Candidate's Dissertation, Chernovtsy (1950).

SYNTHESIS AND PHARMACOLOGY OF MONOMERIC COUMARINS AND THEIR COPOLYMERS

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In view of the diversity of biological activity possessed by the many naturally occurring monomeric coumarin derivatives [1-5], it seems promising to search among them for physiologically active compounds potentially useful as drugs. The poor water solubility of this group of compounds, however, greatly restricts their pharmacological investigation and clinical use. Consequently, the synthesis and investigation of water-soluble coumarin derivatives would be very valuable for practical medicine. As is known, such compounds can be prepared by introducing N-alkyl radicals into the coumarin molecules [5, 6] or by copolymerizing them through the double bond in the 3,4 position of the lactone ring with vinylic comonomers [7-9]. The latter method is the more promising since it greatly prolongs the action of the compounds and in some cases increases it, which is of practical importance.

Proceeding from what has been said in [9, 10], we have synthesized copolymers of coumarin (XX) and a number of its synthetic and natural derivatives substituted in the benzene ring at positions 5, 6,7, 7, and 7,8.

The properties of the monomeric coumarins are indicated in Table 1 and those of their copolymers with N-vinylpyrrolidone (VP) are indicated in Table 2.

It was found that, as with coumarin, polymerization with all of the coumarin derivatives proceeds by a radical mechanism through the double bond in the 3,4 position of the α-pyrone ring. The structure of the resulting polymeric coumarin derivatives was verified on the basis of chemical data and their UV, IR, and NMR spectra [10].

The synthesis of copolymers of VP with coumarins substituted in the α-pyrone ring is of theoretical and practical interest in being a practical method of synthesizing copolymers of

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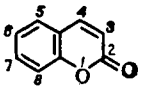
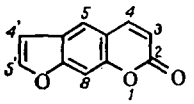
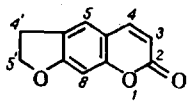
TABLE 1. Monomeric Coumarin Derivatives

No.	Compound	Structure	Melting point, deg	$[\alpha]_D$ deg (solvent)
				
I	Coumarin*	$R_3=R_8=H$	67,5	—
II	3,4-Dihydrocoumarin*	$R_3=R_4=H_2$	—	—
III	Coumarin-3-carboxylic acid	$R_3=COOH$	187	—
IV	4-Hydroxycoumarin*	$R_4=OH$	217—9	—
V	Umbelliferone	$R_7=OH$	231—2	—
VI	Aesculetin*	$R_6=R_7=OH$	271,5	—
VII	Osthole	$R_7=OCH_3$; $R_8=CH_2CH=C(CH_3)_2$	82,5	
VIII	Ostholic acid*	$R_7=OCH_3$; $R_8=CH_2COOH$	251,5	
IX	Merancin hydrate	$R_7=OCH_3$; $R_8=CH_2CHOHC(OH)(CH_3)_2$	127—8	—53,03 (ethanol)
				
X	Isoimperatorin	$R_5=OCH_2CH=C(CH_3)_2$	108,5	
XI	Oxypeucedanin	$R_5=OCH_2CHC(CH_3)_2O$	141,5—2	
XII	Isooxypeucedanin*	$R_5=OCH_2C(=O)CH(CH_3)_2$	145—6	
XIII	Pranferol	$R_5=OCH_2CHOH—CH(CH_3)_2$	108,5—11	—19,38 (chloroform)
XIV	Oxypeucedanin hydrate	$R_5=OCH_2CHOHC(OH)(CH_3)_2$	134—5	
XV	Heraclenin	$R_8=OCH_2CHCCH_3$ (O, CH_3)	103,5—4,5	—22,7 (pyridine)
				
XVI	Marmesin	$R'_5=C(OH)(CH_3)_2$	187,5	+28,2 (chloroform)
XVII	Pranchimgin	$R'_5=C(CH_3)_2OC(O)CH=C(CH_3)_2$	137—8	—30,33 (chloroform)

*These compounds were prepared synthetically. The other tabulated compounds were isolated from various Caucasian Prangos species.

compounds substituted in all positions of the coumarin nucleus, which makes it possible to analyze and draw conclusions about the relation between structure and biological activity for both monomeric and polymeric coumarin derivatives. For this purpose we used two synthetic coumarins, viz. III and IV.

The copolymers of coumarin-3-carboxylic acid (XXXIII) and 4-hydroxycoumarin (XXXIV) were synthesized in ethanol solution in the presence of a radical initiator, azobisisobutyronitrile (ABIBN). Compared with the monomers substituted in the benzene ring, polymerization of the coumarins having substituents in the 3 or 4 position of the α-pyrone ring takes longer and gives copolymers with a very low content of the monomer (about 5 mole %); this is evidently due to steric shielding by the radicals in the α-pyrone ring. The copolymers obtained are white odorless powders soluble in water, physiological saline solution, ethyl alcohol, chloroform, and dimethylformamide (see Table 2).

On the basis of their chemical and spectral data, the following structures can be proposed for the copolymers.

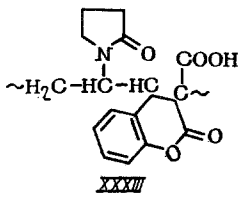
TABLE 2. Copolymers of Coumarin Derivatives with N-Vinylpyrrolidone (VP)

Compound	Nitrogen content, %	Composition, % by weight		Composition, % molar		Yield,* %	Intrinsic viscosity [DMF, 25°], cP
XX (I + VP)	6,47	I	48,6	49,1		0,80	85
XXI (V + VP)	10,0	V	21,0	15,2		0,35	22
XXII (VI + VP)	9,6	VI	23,4	16,0		0,16	26
XXIII (VII + VP)	9,9	VII	21,4	11,0		0,32†	9
						0,50†	12
						0,60†	20
XXIV (VIII + VP)	10,5	VIII	16,7	8,7		0,12	50
XXV (IX + VP)	10,4	IX	17,5	8,0		0,67	36
XXVI (X + VP)	9,8	X	22,3	10,5		0,64	44
XXVII (XI + VP)	8,9	XI	29,0	13,7		0,4†	5
						1,4†	7
XXVIII (XII + VP)	9,8	XII	22,3	10,0		1,20	37
XXIX (XI + VP)	8,9	XI	29,2	13,0		0,25†	6
						1,42†	16
XXX (XIII + VP)	10,0	XIII	20,0	9,0		0,8	34
XXXI (XVI + VP)	9,9	XVI	21,5	11,0		0,25‡	42
XXXII (XVII + VP)	10,8	XVII	14,3	5,4		0,64‡	26
XXXIII (III + VP)	11,77	III	6,6	4,0		0,04	40
XXXIV (IV + VP)	11,7	IV	7,2	5,0		0,2	15

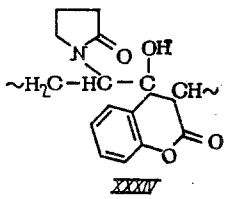
*These are not the maximum possible yields but those to which the intrinsic viscosity values indicated in the next column correspond.

†Fractions obtained by precipitation from ethanol solution with ether.

‡Viscosity measured in water at 25°.



XXXIII



XXXIV

EXPERIMENTAL

Pharmacology

The effect of the compounds on arterial pressure (AP) was determined in acute experiments on 130 rats and 102 cats under urethane narcosis (1 g/kg), and in chronic experiments on 40 rabbits with carotid arteries drawn out into a cutaneous collar and on eight rabbits with experimental renal hypertonia.

The central action of the substances was tested on seven cats, their ganglion-blocking and antiadrenergic action on 62 cats, and their cardiac action on 38 rabbits, while their myotropic action was studied in 154 observations on 25 isolated rabbit auricles. In all experiments the test substances were administered intravenously in equimolar concentrations.

The data obtained were processed statistically according to the Fisher–Student criterion, and differences were regarded as significant at $P \leq 0.05$.

The study of the monomeric coumarin derivatives (see Table 1) in the animal experiments showed their high physiological activity. It was found that compounds I, III, and VI-XVII have a distinct hypotensive effect when administered intravenously. The strength and duration of their hypotensive action depended on the individual characteristics of the compound and the dose administered.

In the acute experiments on cats, a 10 mg/kg dose of monomeric coumarins VII-XVII decreased the arterial pressure by 25-50% of its initial value for 3-6 h; the time for I, III, and VI was 2-5 min, and II, IV, and V did not display hypotensive activity. As is known, hypotensive agents with a long-lasting action are of most value for practical medicine. The duration of the hypotensive action of these compounds cannot be increased by increasing the dose, because they affect heart action and have other side effects at high doses. In view

TABLE 3. Hypotensive Activity of Monomeric Coumarins and Their Copolymers

Compound	Dose (mg/kg) lowering AP by			Duration of hypotonia at AP reduction of			LD_{50}, mg/kg, mouse	
	50%	30%	25%	50%	30%	25%	i.p.	s.c.
I	—	10	—	—	15 min	—	400±20	—
XX	—	20	—	—	40 min	—	—	—
II	—	—	10	—	—	0	—	—
III	—	—	10	—	—	5 min	—	—
XXXIII	—	—	25	—	—	5 min	—	—
IV	—	—	10	—	—	0	—	—
XXXIV	—	—	10	—	—	0	—	—
XXI	—	—	10	—	—	0	—	—
VI	—	—	10	—	—	5 min	—	—
XXII	—	—	25	—	—	10 min	—	—
VII	20	10	—	2 h	1 h	—	—	16
XXIII	50	25	—	4 h	2½ h	—	—	55
IX	25	—	5	2½ h	—	8 h	—	—
XXV	50	—	10	5 h	—	26 h	Nontoxic	
X	50	—	—	1½ h	—	—	—	—
XXVI	100	—	—	3 h	—	—	—	—
XI	5	—	5	3 h	—	7 h	Nontoxic	
XXVII	25	—	20	8 h	—	14 h	The same	
XIII	25	10	—	2 h	4 h	—	—	—
XXX	50	20	—	5 h	7 h	—	—	—
XIV	1,5	—	5	3 h	—	10 h	550±60	—
XXIV	10	—	10	8 h	—	15 h	—	—
XV			5	8 h	—	—	—	—
XVI	30	—	2	2 h	—	12 h	—	—
XXXI	50	—	5	5 h	—	36 h	Nontoxic	
XVII	50	5	—	1½ h	3 h	—	—	72,5
XXXII	100	10	—	3 h	24 h	—	—	87,5

of this, we investigated the copolymers of the substances indicated in Table 1 with VP (XX-XXXIV) (see Table 2). It was found that the hypotensive properties of the coumarins were completely retained after copolymerization with VP, while the duration of the action increased considerably (by a factor of 5 to 10) when equimolar amounts were administered (Table 3).

A study of the mechanism of the hypotensive action of the monomeric coumarin derivatives and their copolymers with VP showed that the reduction in AP is due mainly to the direct myotropic action of the substances.

On the basis of the absence of any specific cholinolytic, antihistamine, or adreno-blocking effects, and data on their spasmolytic action, we can conclude that the test substances have a papaverine-like action on the smooth musculature.

It is of interest that the hypotensive action of the monomers and especially the copolymers shows up clearly in experiments on rabbits with experimental renal hypertonia, the hypotension lasting 5-10 times longer after administration of the copolymers than after administration of the monomers.

Some of the copolymers (XXIII, XXVII and XXIX) display antiarrhythmic activity, which was studied on models of arrhythmia induced by calcium chloride, aconitine, strophanthine, adrenaline and electric stimulation, and compared with the antiarrhythmic activity of novocainamide (XXXVI), quinidine (XXXVII) and propranolol (XXXVIII). It was found that the copolymers show their highest antiarrhythmic activity at doses of 45-50 mg/kg, and that their antiarrhythmic index is two to four times higher (10-20) than that of novocainamide (5.5), which is in wide clinical use as an antiarrhythmic agent. In addition, a comparison of the data obtained by studying a number of fractions of XXIII, XXVII and XXIX with different viscosity characteristics (see Table 2) showed that the antiarrhythmic activity of these compounds increases with their molecular weight (Table 4).

It should be noted that there is no single opinion about the central action of coumarin derivatives in the literature. We therefore paid special attention to this question. Thus, our experiments on mice indicated that some of the natural substances have a depressant

TABLE 4. Antiarrhythmic Activity of Copolymers of Some Natural Coumarins (50 mg/kg)

Compound	Viscosity, cP	Form of arrhythmia							
		effectiveness from 7-11 observations, %			adrenaline, on chloroform-narcotized cats, % protection	Strophanthine, on piglets		electric stimulation, cats	
		calcium, on rats	aconitine, on rats			protection, %	prolongation of life	auricle	auricle and ventricle
		protection	protection	relief					
XXIII	0,60	0	0	0	—	—	—	—	—
XXIII	0,50	43	0	0	—	—	—	—	—
XXIII	0,32	43	10	10	—	—	—	—	—
XXVII	1,4	35	35	25	—	—	—	—	—
XXVII	0,4	50	65	55	54 (±11 min)*	21 (±3min)*	45 min	Complete protection	Complete protection
XXIX*	0,42	40	60	45	40 (±8,5min)	35 (±5 min)	50 min	Ditto	Ditto
XXIX*	0,25	60	75	65	60 (±12min)	48 (±7 min)	80 min	"	"
Novocainamide, 20 mg/kg	—	25	0	20	15 (±2min)	10 (±1 min)	21 min	12±1 min	15 min
Quinidine, 3 mg/kg	—	0	10	0	—	—	—	15±2,5 min	0
Propranolol, 2 mg/kg	—	—	—	—	18 (±2,5min)	17 (±2,5 min)	33 min	—	—

Note. All compounds were administered intravenously; —) experiment not performed; 0) effect not noted.
*Maximum dose 30 mg/kg.

effect on the animals' cerebral structures. Thus, when administered at a dose of 1 mg/kg in combination with barbamyl (80 mg/kg), compounds X, XI, and XIV greatly prolong the hypnotic effect of the former (from 3-5.5 to 120-180 min). The same compounds also increase the duration of the hypnotic effect of hexenal by approximately 50%. For example, at a dose of 10 mg/kg, XIV increased the duration of hexenal-induced sleep on average from 70 to 100 min, XI increased it from 25 to 80 min, and X increased it from 55 to 100 min. However, none of the investigated natural coumarins potentiated the hypnotic effect of chloral hydrate at a dose of 10 mg/kg.

Registration of the bioelectric activity of the brain of rabbits with chronically accustomized electrodes also showed that VII, X, XI and XIV did not alter the background bioelectric activity of the brain at doses of 1, 5 and 10 mg/kg. Neither did analog processing of the EEG reveal any change: On average, the signal recurrence frequency remained the same as the number of signals in the control period.

It is known that the mechanism by which various substances potentiate hypnotic action may involve inhibition of barbiturate-destroying microsomal enzymes in the liver, a sedative effect, or a combination of these mechanisms.

By comparing the results obtained in experiments on mice using barbamyl and chloral hydrate, and also hexenal, which undergoes conversion in the liver, with data from experiments with medinal, which is secreted in unchanged form from the organism, we reached the conclusion that the coumarin derivatives can prolong the effect of hypnotics that undergo conversion in the liver. We can assume, therefore, that this group of compounds blocks the microsomal systems of the liver that are involved in the inactivation of the hypnotics.

At high doses (80-100 mg/kg) the test substances have a depressant action on the animals. Thus, when XIV was administered at a dose of 50-80 mg/kg, we noted a short-lived sharp drop in respiration, which then became superficial and partial, relaxation of the musculature, twitching, and sometimes death (usually on rapid administration). At the same time we observed a change in the EEG: The background activity was replaced by a high-amplitude rhythm on administration of XI and XIV, while the opposite changes were observed in the case of VII and X (80 mg/kg). After administration of VII, for example, the EEG showed a pronounced activation reaction, which was retained for 2 h.

On the basis of an analysis of the experimental data on the pharmacological action of the test compounds, we can form several conclusions about the relation between their structure and biological activity. It should first be noted that the most active of the group of substances investigated (see Table 1) were the furocoumarins substituted in the 5 and 8 positions of the benzene ring (for example, XI, XIV, their copolymers, and XV) (see Table 3), their hypotensive activity varying with the structure of the side chain, i.e., from 2,3-epoxyisopentyloxy (XI, XV) to 2,3-dihydroxyisopentyloxy (XIV). As can be seen, the change from an epoxy group to hydroxy groups leads to an increase in biological activity, while substances with two hydroxy groups in the side chain (XIV) are more active than those with one hydroxy group (XIII) (see Table 3). The least active of the furocoumarins investigated was X, which contains an isopenten-2-yloxy group. Compound XVI, which contains a $C(OH)(CH_3)_2$ group in the 5' position, is more active than XVII, in which the hydroxy group is esterified by a senecioic acid residue. The same picture is observed in the case of the copolymers of these compounds.

The most active of the coumarins proved to be VII and IX, which are substituted in the 7 and 8 positions and contain isopentyl and 2,3-dihydroxyisopentyl groups, respectively. As can be seen from the data reported, the transition from an isopentyl to a dihydroxyisopentyl radical does not significantly alter the activity of these compounds.

Compounds I, III and VI display only a short-lived hypotensive action (2-5 min), and II, IV, and V do not possess this property at all. It is interesting that the copolymer of coumarin-3-carboxylic acid (XXXIII), unlike the other copolymers (XX-XXXII), does not have a prolonged action.

Chemistry

The UV spectra were recorded with a Perkin-Elmer 402 spectrophotometer in ethanol. The PMR spectra were obtained using a Bruker XH-90 spectrometer.

The synthesized compounds were chromatographed on Silufol UV-254 plates in chloroform. The working solutions of the test substances were prepared by dissolving them in dimethyl sulfoxide, water, on Tween-80 with addition of water.

Coumarin-3-carboxylic Acid (III). A mixture of 20 ml (0.14 mole) of salicylaldehyde and 15 g (0.14 mole) of malonic acid was stirred and treated with 10 ml of pyridine, and heated on a waterbath at 80-85°C until a homogeneous mass was obtained. After cooling, the reaction mixture was treated with 6 N hydrochloric acid to pH 3.0-4.0. The resulting voluminous precipitate was filtered off and recrystallized three times from ethanol to give a crystalline substance with a mp of 186-187° (187-188° according to [11]; R_f = 0.15, identical to coumarin-3-carboxylic acid. Yield 12 g (80%). UV spectrum: λ_{max} 290 nm; PMR spectrum (in $CDCl_3$; TMS as internal standard; δ, ppm): 8.77 (1H, singlet, COOH), 7.29-7.62 (5H, multiplet, H-4 to H-8).

Copolymer XXXIII. A mixture of 3.3 g VP, 0.19 g III, 0.007 g ABIBN, and 4 ml ethanol was heated in a sealed ampul in a nitrogen stream at 70° in a thermostat for 10 days, after which the ampul was opened and the resulting copolymer XXXIII precipitated from ethanol with ether. The product was dried under vacuum. The purification of XXXIII was monitored by thin-layer chromatography, which indicated the absence of III in the copolymer. Yield 1.36 g (40%). UV spectrum: λ_{max} 277 nm. Copolymer XXXIII is readily soluble in water, alcohol, and dimethylformamide.

Copolymer XXXIV. An ampul was charged with 11.1 g VP, 0.81 g IV, 0.024 g ABIBN, and 12 ml ethanol. The ampul was sealed in a stream of nitrogen and heated in a thermostat at 70° for 30 days. The resulting copolymer XXXIV was precipitated from ethanol with ether and dried under vacuum. Yield 1.86 g (15%). UV spectrum: λ_{max} 272, 284 nm. Copolymer XXXIV was chromatographically pure and readily soluble in both water and organic solvents.

LITERATURE CITED

1. T. O. Soine, J. Pharm. Sci., 53, 231 (1964).
2. P. K. Bose et al., Ind. Chem. Soc., 35, 367 (1958).
3. B. E. Nielsen, Dan. Tidssk. Farm., 44, No. 6, 111 (1970).
4. I. Ya. Khodzhai, Tr. Biol. Inst. Novosibirsk. Otd. Akad. Nauk SSSR, No. 12, 25 (1965).
5. J. Sato et al., Chem. Pharm. Bull., 20, 905 (1972).
6. J. Sato et al., Chem. Abstr., 74, 22696m (1971).
7. E. C. Chapin and R. J. Longley, US Patent No. 2809187 (1957); Ref. Zh. Khim., 20152p (1960).
8. E. Gipstein and W. Hewett, Macromolecules, 2, No. 1, 82 (1969).
9. O. S. Karchmarik, A. Z. Abyshev, I. V. Brodskii, et al., Inventor's Certificate No. 316703 (1971); Otkrytiya, No. 30, 82 (1971).
10. A. Z. Abyshev, P. P. Denisenko, I. V. Brodskii, et al., Khim. Geterotsikl. Soedin., No. 6, 714 (1974).
11. L. L. Woods and J. Sapp, J. Org. Chem., 30, 312 (1965).