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SYNTHETIC ANTIGENS BASED ON POLYMERIC COUMARIN DERIVATIVES

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The paper gives the results of the synthesis and a study of the structure of copolymers of 14 coumarin derivatives with N-vinylpyrrolidone. Using physicochemical methods, their characteristic viscosities, molecular masses, and chemical compositions have been determined. As the result of the immunological investigation performed, it has been shown that some of them possess antigenic properties. Thus, a new type of synthetic antigen has been created.

We set ourselves the aim of obtaining synthetic polymeric compounds possessing the properties of full-value antigens from chemical substances foreign to the organisms. Our attention was attracted by copolymers of certain vinyl derivatives and, in the first place, N-vinylpyrrolidone with coumarin derivatives. Such copolymers of suitable molecular mass are readily formed under the conditions of radical polymerization at a relatively low temperature — 70-80°C — in the presence of an initiator of the radical type — azoisobutyronitrile (AIBN). It should probably be pointed out that a number of the copolymers mentioned possess pronounced prolonged antiarrhythmic and hypotensive action [1, 2], and some of the coumarins composing them exhibit a photosensitizing effect [3, 4]. The latter can be explained by an allergization of the organism, which underlines the probability of the immunoactivity of such compounds.

The results given enabled us to suggest that some of these polymers would prove to be immunogenic.

To solve the problem we synthesized copolymers of the following coumarin derivatives — obtusifol acetate (I), obtusin (II), latilobinol (III), isosamarcandin (IV), grandivitin (V), the methyl ether of khellactone (VI), xanthyletin (VII), and synthetic analogs of them — 6-carboxy-7-(1'-carboxyisopropoxy)coumarin (VIII), oxypeucedanic acid (IX), 8-(3'-chloro-3'-methylbutyl)-7-methoxycoumarin (X)*, 7-(2'-bromoethoxy)coumarin (XI)*, 7-(2'-bromoethoxy)-4-methylcoumarin (XII), 7-(3'-chloropropoxy)coumarin (XIII), and 7-(3'-chloropropoxy)-4-methylcoumarin (XIV) with N-vinylpyrrolidone (scheme).

*These copolymers were obtained with the cooperation of I. V. Brodskii and V. A. Kropachev.

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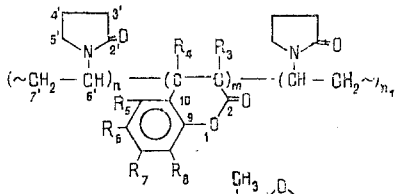
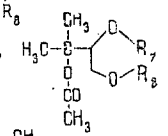
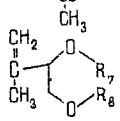
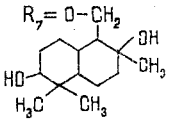
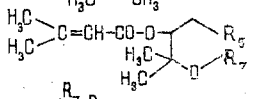


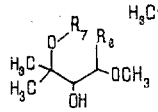
I. $R_3 = R_4 = R_5 = H$, $R_6 = OCH_3$, 

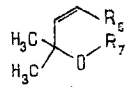
II. $R_3 = R_4 = R_5 = H$, $R_6 = OCH_3$, 

III. $R_3 = R_4 = R_5 = R_6 = R_8 = H$, $R_7 = O-CH_2-CH=C(CH_3)-CH_2-CH_2-$

IV. $R_3 = R_4 = R_5 = R_6 = R_8 = H$, $R_7 = O-CH_2-$ 

V. $R_3 = R_4 = R_5 = R_8 = H$, 

VI. $R_3 = R_4 = R_5 = R_6 = H$, 

VII. $R_3 = R_4 = R_5 = R_8 = H$, 

VIII. $R_3 = R_4 = R_5 = R_8 = H$, $R_6 = COOH$, $R_7 = O-C(CH_3)_2-COOH$

IX. $R_3 = R_4 = R_8 = H$,  , $R_5 = O-CH_2-COOH$

X. $R_3 = R_4 = R_5 = R_6 = H$, $R_7 = OCH_3$, $R_8 = -CH_2-CH_2-C(CH_3)_2Cl$

XI. $R_3 = R_4 = R_5 = R_6 = R_8 = H$, $R_7 = -O-CH_2^{11}-CH_2^{12}-Bz$

XII. $R_3 = R_5 = R_6 = R_8 = H$, $R_7 = -O-CH_2-CH_2-Bz$, $R_4 = -CH_3$

XIII. $R_3 = R_4 = R_5 = R_6 = R_8 = H$, $R_7 = -O-CH_2-CH_2-CH_2-Cl$

XIV. $R_3 = R_5 = R_6 = R_8 = H$, $R_7 = -O-CH_2-CH_2-CH_2-Cl$, $R_4 = -CH_3$

The polymeric coumarin derivatives mentioned consist of strong statistical carbochain polymers the chemical structures of which are easy to check by modern physicochemical methods — UV, IR, PMR, and ^{13}C NMR spectroscopy. Some of the parameters of the copolymers synthesized are given in Tables 1 and 2.

As an example, we shall discuss the results of the study of the structure of (XI), as one of the synthetic antigens.

The IR spectrum of (XI) had absorption bands at 1770 cm^{-1} (C=O of an α-dihydropyrone ring), 1695 (C=O in the vinylpyrrolidone ring), and 1590 and 1510 cm^{-1} (—CH=CH— bond in an aromatic ring). The presence in the spectrum of (XI) of a band at 1770 cm^{-1} showed that copolymerization had taken place at the 3,4-double bond of the α-pyrone ring of the coumarin nucleus. This was also confirmed by the PMR spectrum of (XI) in which in the weak-field region there were only the signals of the protons of aromatic rings (broadened signal at 7.10 ppm, H-5, H-6, and H-8), and the signals of the protons of the —CH—CH— grouping in the α-pyrone ring were shifted upfield and appeared at 2.40-3.70 ppm.

In the ^{13}C NMR spectrum of (XI) the signals of the carbon atoms of each comonomer — N-vinylpyrrolidone (XV) and 7-(2'-bromoethoxy)coumarin (XVI) — appeared (Table 3). The assignment of these signals was easily done by comparing the ^{13}C NMR spectra of the monomers and their copolymers. The only exceptions were the signals of the carbon atoms in positions 3, 4, 6', and 7', which, in the spectrum of (XI) appeared in a stronger field, this being due to a change in the degree of hydridization of these carbon atoms in the copolymerization process. Thus, it can be seen that the physicochemical characteristics given agree completely with the structure (XI).

TABLE 1. Compositions of Copolymers of Vinylpyrrolidone with Coumarin Derivatives

Number of the copolymer	Composition of the initial mixture*					Polymerization time	Yield (total) on the charge		N, %	Composition of the copolymer, %			
										by weight		molar	
	M_1, g	M_2, g	$M_1:M_2$	AIBN, g	solvent, ethanol, ml		g	%		M_1	M_2	M_1	M_2
I	2,22	0,5	13:1	0,01	10	1h 20m	0,8	29,41	9,5	75,39	24,61	90,22	9,78
II	5,5	0,75	17:1	0,015	8	3 h	1,50	24,0	9,8	77,77	22,23	89,62	10,38
III	5,0	0,5	50:1	0,005	10	55 m	0,85	15,45	8,95	71,03	28,97	89,41	10,59
IV	11	0,8	50:1	0,05	12	1,5 h	1,2	10,17	9,75	77,38	22,62	92,50	7,50
V	4,0	0,72	13:1	0,01	8	1,5 h	2,30	48,72	8,8	69,84	30,16	82,65	17,35
VI	7,5	0 5	34:1	0,02	15	25 m	3,20	40,00	10,5	83,33	16,67	93,75	6,25
VII	3,5	0,45	19:1	0,09	5	40 m	2,55	64,55	10,8	85,71	14,29	93,72	6,28
VIII	2,2	0,25	22:1	0 015	5	1 h	0,8	32,65	8,9	70,63	29,37	86,48	13,52
IX	1,5	0,5	5:1	0,05	10	6 h	1,5	75,0	9,8	77,77	22,23	89,11	10,89
X	20,0	1,2	42:1	0,02	24	50 m	7,0	33,02	10,1	84,16	15,84	92,0	8,0
	4,0	0,8	13:1	0,02	10	1,5 h	3,2	93,75		76,8	23,2	87,9	12,1
	7 14	0,36	50:1	0,2	20	40 m	5,0	75,0		93,2	6,8	97,2	2,8
XI	22,2	1,34	40:1	0,05	10	50 m	4,2	18,0	9,7	85,80	14.19	93,62	6,38
	5,55	0,67	20:1	0,01	5	1h 35m	2,33	37,45		71,3	28,7	85,7	14,3
	11,0	0,62	4:1	0,2	15	1h 30m	3,5	82,0		85,8	14,2	90,1	9,9
XII	4,4	0,85	13:1	0,05	5	52 h	1,5	28,6	9,5	75,4	24,6	88,31	11,69
XIII	8,5	2,5	8:1	0,1	10	2,5 h	4,5	41,0	8,9	70,63	29,37	84,21	15,79
XIV	15,0	8,0	5:1	0,25	15	85 h	12	52,0	10,5	83,33	16,67	91,46	8,54

*For convenience, the comonomers — N-vinylpyrrolidone and the coumarin derivatives — are denoted by M_1 and M_2, respectively. The compositions of the copolymers were calculated from the amounts of nitrogen in them on the basis of the percentage of nitrogen in pure vinylpyrrolidone, which is 12.6%. In addition, it is not the maximum possible yields that are shown but those to which the values of the characteristic viscosities and molecular masses given in Table 2 correspond. In some cases, the compositions of halogen-containing copolymers were calculated on the basis of their Cl or Br contents.

The antigenic properties of the copolymers (I-XIV) were determined at our request in the Institute of Toxicology of the Ministry of Health of the USSR (M. P. Podosinovikova) and in the Leningrad Institute for The Advanced Training of Doctors (L. F. Shabanova) using the gnerally adopted schemes of immunization on rabbits. As a result, of the investigation performed, it was shown that, among the polymeric coumarins studied, (X) and (XI) possessed the most pronounced antigenic properties. The antisera obtained on immunization with copolymer (XI) had titers of from 1/8-32 to 1/128, and for the copolymer (X) the negative logarithm of the titer on the seventh day of immunization was 2.4 and on the 14th day 3.0. Thus, it was shown that copolymers (X) and (XI) possessed antigenic properties, i.e., the animal organism is capable of forming antibodies in response to the injection of these compounds. Our hypothesis of the possible immunogenicity of some copolymers of vinyl derivatives and, in particular, N-vinylpyrrolidone, with coumarin derivatives has been confirmed.

No antigenic properties have previously been described for compounds of this series. Consequently, it can be stated that we have created a new type of synthetic antigen. An original feature of such antigens is the fact that they are constructed wholly from compounds uncharacteristic of (foreign to) the animal organism. We assume that the role of antigenic determinants in the polymeric coumarins studied is played by the structural components of the differently substituted coumarin derivatives. In this connection, it must specially be emphasized that the antigenic properties of the copolymers (X) and (XI) that we synthesized are due mainly to the presence in their molecules of aromatic rings condensed with an α-pyrone ring and of halogens, to the rigidity of their chemical structure, and to the stability of the configurations of the coumarin units in the polymer chain. The antigenicity of these copolymers also depends on their chemical composition, i.e., the number of coumarin units introduced into the macromolecule. With an increase in the number of coumarin units in the macromolecules of the copolymers, their immunogenicity increases.

Of definite interest in relation to our investigations on the possibility of obtaining synthetic antigens from compounds foreign to the organism is the work of A. M. Nadzhitmit-

TABLE 2. Some Parameters of the Copolymers (I-XIV)

Number of the copolymer	IR spectra, CO, cm^{-1}	PMR spectra*, δ, ppm	$[\eta]$ or M_η
I	1772, 1770	6,92 (H-5), 3,80 $-OCH_3$	0,85 38000
II	1768, 1695	3,80 $-OCH_3$, 6,50 (H-5), 1,75; 5,35 $-C(CH_3)=CH_2$	0,75 35000
III	1765, 1705	1,0 $-C(CH_3)_2-CH_3$, 1,80 $=C-CH_3$, 4,90 $=CH_2$ and $-O-CH_2-$, 7,0 (H-5, H-6, H-8)	0,29 22000
IV	1770, 1700	1,10 $-C(CH_3)_2-CH_3$ and $-C(CH_3)_2-O$, 4,75 $-O-CH_2-$, 6,95 (H-5, H-6 and H-8)	0,67
V	1775, 1685	1,20 $-C(CH_3)_2-CH_3$, 6,85 (H-5 and H-8), 4,85 $-CH-CO-O-$, 5,30 $=CH-$	0,68
VI	1770, 1695	1,20 $-C(CH_3)_2-CH_3$, 6,80 (H-5 and H-6), 3,95 $-OCH_3$	0,59
VII	1708, 1677	1,25 $-C(CH_3)_2-CH_3$, 6,75 (H-5, H-8, H-3′ and H-4′)	0,62
VIII	1765, 1685	6,80 (H-5 and H-8), 9,50 COOH	0,45
IX	1770, 1680	5,0 $-O-CH_2-$, 6,80 (H-8), 6,95 (H-5), 7,40 (H-4′), 9,75 $-COOH$	0,62
X	1770, 1695	4,0 $-OCH_3$, 6,62 (H-5 and H-6)	0,75 48000
XI	1770, 1695	4,40 $-O-CH_2-$, $-CH_2-Br$, 7,10 (H-5, H-6 and H-8)	0,65 20000
XII	1775, 1700	2,40 $=C-CH_3$, 4,30 $-O-CH_2-$, $-CH_2-Br$, 6,95 (H-5, H-6 and H-8)	0,60 32000
XIII	1775, 1695	3,88 $-CH_2-Cl$, 4,20 $-O-CH_2-$, 7,0 (H-5, H-6 and H-8)	0,35 25000
XIV	1768, 1685	2,32 $=C-CH_3$, 3,65–4, 10 $-O-CH_2-$, $-CH_2-Cl$, 6,95 (H-5, H-6 and H-8)	0,82 42000

*The signals of the protons of vinylpyrrolidone and the α-pyrone ring in the copolymers appeared in all cases at 1.90-2.20, 3.20, and 3.66 ppm. Consequently, only the chemical shifts of the signals due to the protons of the aromatic moiety of the molecule and to individual fragments of the substituents are given.

dinov et al. [5], in which it has been shown that copolymers of N-vinylpyrrolidone with acrylic acid having a molecular mass of 300,000-400,000 are antigens for mice.

In conclusion, two important features of copolymers (X) and (XI) must be mentioned: 1) In doses of up to 5000 mg/kg they exhibit no toxic action; and 2) the copolymers (X) and (XI) contain fairly reactive groups which will permit their use as carrier for the production of artificial antigens.

EXPERIMENTAL

IR spectra were taken on a UR-20 spectrometer (in paraffin oil), and PMR and ^{13}C NMR spectra on a HX-90 spectrometer (in $CDCl_3$; 0 — TMS). Molecular masses were determined by

TABLE 3. Assignment of the Signals in the ^{13}C NMR Spectrum of N-Vinylpyrrolidone (XV), 7-(2'-Bromoethoxy)coumarin (XVI), and the Copolymer (XI)

Carbon atom	XV	XVI	XI
C-2		160,9	166,8
C-3		112,7	45,0–41,0
C-4		142,9	45,0—41,0
C-5		128,8	129,7
C-6		113,5	111,0
C-7		162,2	157,9
C-8		101,7	102,9
C-9		156,1	150,6
C-10		112,7	115,6
C-11		68,2	68,1
C-12		28,4	28,8
C-2′	176,0		174,8
C-3′	31,8		31,3
C-4′	17,6		18,4
C-5′	45,6		42,0
C-6′	129,5		45,0—41,0
C-7′	96,4		36,0—30,0

the sedimentation method on a Beckman Model E analytical centrifuge. Characteristic viscosities were determined with the aid of an Ubbelohde viscometer in dimethylformamide at 25°C. The nitrogen contents of the copolymers were determined in a Hewlett-Packard automatic C, H, N analyzer. The fractionation of the initial samples of copolymers (X) and (XI) was carried out by the fractional precipitation method.

The copolymerization of vinylpyrrolidone with coumarin derivatives was carried out in sealed tubes under conditions described in the literature [2, 3].

SUMMARY

Copolymers of N-vinylpyrrolidone with coumarin derivatives have been synthesized which possess antigenic properties.

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