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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF DERIVATIVES OF PHOSPHORUS- AND NITROGEN-CONTAINING COUMARINS

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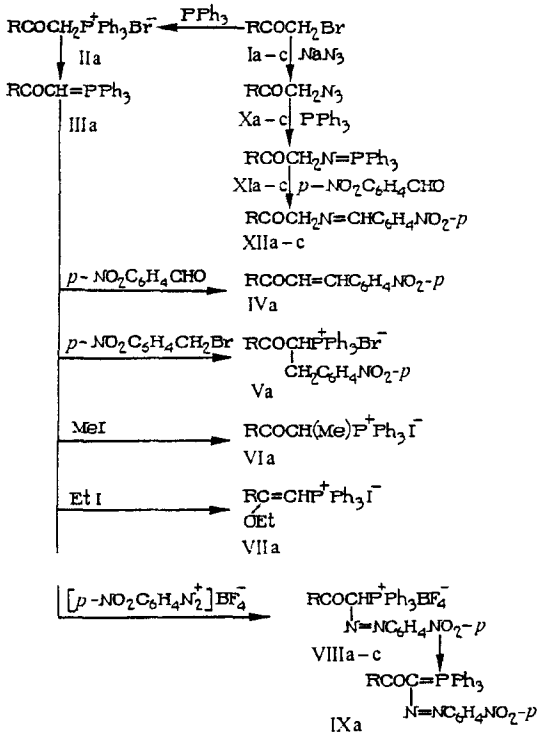
A number of P- and N-containing derivatives of coumarins were synthesized. The studied substances were shown to exhibit antibacterial and antifungal activity.

It was shown previously that a number of compounds containing various aromatic and heterocyclic systems in position 3 of the coumarin ring have antibacterial activity [1 – 3].

Continuing the work on the synthesis, and study of the properties and biological activity of phosphonium and ammonium derivatives of coumarin [4], we synthesized phosphorus- and nitrogen-containing systems of coumarins, 5,6-benzocoumarins and studied their antimicrobial activity.



The starting compound for synthesizing compounds (IIa – IXa) was 3-(bromacetyl)-5,8-dichlorocoumarin (Ia) prepared by bromination of 3-acetyl-6,8-dichlorocoumarin in a dioxane–ether mixture. The reaction of compound Ia with triphenylphosphine in a benzene solution yields the phosphonium salt IIa that smoothly dehydrobrominates when an ethanol solution is treated with an aqueous potassium carbonate solution. The resulting phosphorus ylide IIIa readily reacts both with *p*-nitrobenzaldehyde (to form the 6,8-dichlorocoumarin-containing unsaturated ketone IVa) and with some alkylating agents. When IIIa is heated with MeI and *p*-nitrobenzyl bromide, C-alkylation occurs (see structures Va – VIa); when EtI is used, the O-alkylated product VIIa is obtained. Electrophilic addition of *p*-nitrophenyldiazonium borofluoride to the P-C bond of phosphorylides IIIa – c occurred. When a dimethylformamide solution of the phosphonium salt of VIIIa is treated with an aqueous potassium carbonate solution, the corresponding phosphorus ylide IXa forms.

To prepare coumarin compounds (XIa-c) containing a triphenylphosphazo group, we used the reaction of 3-(azidoacetyl)-coumarins (Xa – c) with triphenylphosphine. Compound Xa – c was synthesized by reacting the relevant compounds Ia – c with sodium azide. The reaction of the phosphazo compounds XIa – c with *p*-nitrobenzaldehyde yielded the azomethines (XIIa – c).

R-6,8-dichlorocoumarinyl-3 (a), coumarinyl-3 (b), or 5,6-benzocoumarinyl-3 (c).

Compounds Ib, c with sodium thiocyanate in methanol form XIIIb, c. Heterocyclization of XIIIb in acetic and sulfuric acids and of the 5,6-benzocoumarin-containing unsaturated ketone XVc with thiourea yielded the corresponding thiazolone XIVb and 2-thioxopyrimidine XVIc.

The structure of the synthesized compounds is confirmed by the IR spectra.

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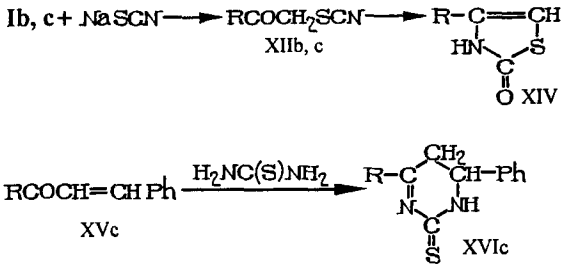

CHEMICAL EXPERIMENTAL PART

The IR spectra were obtained in KBr pellets on a Specord IR-75 spectrophotometer. The synthesized compounds are characterized in Table 1. The found values of the elemental analyses correspond to the calculated values.

3-(Bromacetyl)-6,8-dichlorocoumarin (Ia). A solution of 2.57 g (0.01 mole) of 3-acetyl-6,8-dichlorocoumarin in 20 ml of ether and 30 ml of dioxane at 30°C was added with 0.52 ml of bromine dropwise with stirring. We then stirred the solution for one hour. We filtered the precipitate, washed it with water, and dried it.

2-(6,8-Dichlorocoumarin-3-yl)-2-oxoethyltriphenylphosphonium bromide (IIa). We added a solution of 1.31 g (0.005 mole) of triphenylphosphine in 10 ml of benzene to a solution of 1.67 g (0.005 mole) of compound Ia in 20 ml of benzene. We boiled the reaction mixture for one hour. We separated the crystalline precipitate, washed it with ether, and dried it. IR spectrum, ν_{max}, cm^{-1}: 1630 (CO).

2-(6,8-Dichlorocoumarin-3-yl)-2-oxoethylidenetriphenylphosphorus ylide (IIIa). To a solution of 1.5 g (0.0025 mole) of the phosphonium salt of IIa in 15 ml of ethanol, we added with stirring 10 ml of a 10% aqueous solution of potassium carbonate. We let the solution with the precipitate stand for 12 h at room temperature. We separated compound IIIa, washed it with water and dried it. IR spectrum, ν_{max}, cm^{-1}: 1392 ("P-C absorption I"), 890 ("P-C absorption II").

1-(6,8-Dichlorocoumarin-3-yl)-3-(4-nitrophenyl)propen-1-one (IVa). To a solution of 1 g (0.0017 mole) of phosphorus ylide IIIa in 15 ml of toluene, we added a solution of 0.3 g (0.002 mole) of *p*-nitrobenzaldehyde in 5 ml of toluene. We heated the reaction mixture for four hours with a reflux condenser. IR spectrum, ν_{max}, cm^{-1}: 1675 (CO), 1625 (C–C). Compound IVa is a trans-isomer, which is confirmed by the presence of a band at 1010 cm^{-1}, due to the bending vibrations of the =C–H trans-substituted vinylene group [5].

2-(6,8-Dichlorocoumarin-3-yl)-1-(p-nitrobenzyl)-2-oxoethyltriphenylphosphonium bromide (Va). We heated 1.29 g (0.0025 mol) of phosphorus ylide IIIa and 0.54 g (0.0025 mol) of *p*-nitrobenzyl bromide in 15 ml of anhydrous toluene for 10 h with a reflux condenser without allowing access to atmospheric moisture. We filtered off the phosphonium salt that precipitated when the solution was cooled, and washed it with ether. IR spectrum, ν_{max}, cm^{-1}: 1655 (CO).

2-(6,8-Dichlorocoumarin-3-yl)-1-methyl-2-oxoethyltriphenylphosphonium iodide (VIa). We heated 1.29 g of phosphorus ylide IIIa and 7 ml of MeI for two hours. We separated the precipitate and washed it with ether. IR spectrum, ν_{max}, cm^{-1}: 1640 (CO).

2-(6,8-Dichlorocoumarin-3-yl)-2-ethoxyethenyltriphenylphosphonium iodide (VIIa). We heated 1.29 g of phosphorus ylide IIIa and 7 ml of EtI with a reflux condenser for three hours. After evaporation of the surplus, we ground the obtained oil with 15 – 20 ml of hexane until complete crystallization.

2-(6,8-Dichlorocoumarin-3-yl)-1-(*p*-nitrophenylazo)-2-oxoethyltriphenylphosphonium borofluoride (VIIIa). We heated a mixture of 0.0025 mol of phosphorus ylide IIIa and 0.0025 mole of *p*-nitrophenyldiazonium borofluoride in 10 ml of acetonitrile for three hours. We distilled off the solvent at a reduced pressure. We ground the oily residue with hexane until complete crystallization.

Using the appropriate phosphorus ylides [6, 7], we synthesized compounds VIIIb, c by a similar procedure.

2-(6,8-Dichlorocoumarin-3-yl)-1-(*p*-nitrophenylazo)-2-oxoethylidenetriphenylphosphorus ylide (IXa). To a solution of 0.94 g (0.0012 mole) of compound VIIIa in 5 ml of DMF, we added 5 ml of a 10% potassium carbonate solution

TABLE 1. Characteristics of Synthesized Compounds

Compound	Yield, %	M. p.,°C (solvent)	Molecular formula
Ia	61	193-5 (AcOH)	$C_{11}H_5BrCl_2O_3$
IIa	82	180-4 (decomp.) (ethanol-butyl acetate, 1 : 1)	$C_{29}H_{20}BrCl_2O_3P$
IIIa	98	243-5 (toluene)	$C_{29}H_{19}Cl_2O_3P$
IVa	60	251-3 (toluene)	$C_{18}H_9Cl_2NO_5$
Va	50	168-72 (decomp.) ($CHCl_3$-ether, 1 : 1)	$C_{36}H_{25}BrCl_2NO_5P$
VIa	91	215-7 (butanol)	$C_{30}H_{22}Cl_2IO_3P$
VIIa	97	125-7 ((butanol-ether, 1 : 1)	$C_{31}H_{24}C_{12}IO_3P$
VIIIa	74	160-2 ((butanol)	$C_{35}H_{23}BCl_2F_4N_3O_5P$
VIIIb	85	176-8 (butanol)	$C_{35}H_{25}BF_4N_3O_5P$
VIIIc	95	132-4 (butanol)	$C_{39}H_{27}BF_4N_3O_5P$
IXa	65	185-7 (butanol)	$C_{35}H_{22}Cl_2N_3O_5P$
Xa	80	295-7 (ethanol)	$C_{11}H_5Cl_2N_3O_3$
Xb	72	243-5 (ethanol)	$C_{11}H_7N_3O_3$
Xc	90	282-4 (ethanol)	$C_{15}H_9N_3O_3$
XIa	70	208-10 ($CHCl_3$-ether, 1 : 1)	$C_{29}H_{20}Cl_2NO_3P$
XIb	82	178-80 ($CHCl_3$-ether, 1 : 1)	$C_{29}H_{22}NO_3P$
XIc	78	205-7 ($CHCl_3$-ether, 1 : 1)	$C_{33}H_{24}NO_3P$
XIIa	43	232-4 (nitromethane)	$C_{18}H_{10}C_{12}N_2O_5$
XIIb	95	216-8 (nitromethane)	$C_{22}H_{14}N_2O_5$
XIIc	85	196-8 (nitromethane)	$C_{22}H_{14}N_2O_5$
XIIIb	87	156-8 (dioxane)	$C_{12}H_7NO_3S$
XIIIc	82	168-70 (dioxane)	$C_{16}H_9NO_3S$
XIVb	81	222-4 (acetoacetic ester)	$C_{12}H_7NO_3S$
XVc	88	153-5 (toluene)	$C_{22}H_{14}O_3$
XVIa	30	> 340 (methanol)	$C_{23}H_{16}N_2O_2$

TABLE 2. Minimal Inhibiting Concentrations (in μg/ml of medium) Inhibiting Microorganism Growth

Compound	*E. coli* 12	*S. aureus* 209	*P. aeruginosa* 40	*B. subtilis* 39	*C. albicans* 23	S. cerevisiae
IIa	500	125	500	250	250	15,6
IIIa	31.2	< 3.9	250	n/a	< 3.9	< 3.9
IVa	500	250	n/a	n/a	500	31.2
Va	500	62,5	n/a	n/a	250	125
VIa	500	125	500	n/a	250	0.9
VIIa	500	125	500	n/a	500	62.5
VIIIa	500	62.5	500	n/a	250	62.5
VIIIb	500	15.6	n/a	n/a	250	31.2
VIIIc	n/a	62.5	n/a	n/a	250	62.5
IXa	500	< 3.9	n/a	n/a	125	250
Xa	500	< 3,9	500	n/a	62.5	62.5
Xb	500	125	500	n/a	250	15.6
Xc	500	125	500	n/a	250	125
XIa	n/a	62.5	500	n/a	250	125
XIb	500	15.6	500	n/a	500	62.5
XIc	500	125	500	n/a	500	62.5
XIIa	n/a	250	500	250	125	125
XIIb	500	7.8	500	< 3.9	250	62.5
XIIc	500	31.2	500	125	250	250
XIIIb	n/a	250	500	250	250	125
XIIIc	n/a	< 3.9	500	n/a	15.6	7.8
XIVb	n/a	< 3.9	n/a	n/a	125	125
XVIc	500	< 3.9	n/a	< 3.9	< 3.9	< 3.9

Note. n/a signifies that the compound in the tested concentrations is not active.

while stirring. We separated the precipitate, washed it with water, and dried it.

3-(Azidoacetyl)-6,8-dichlorocoumarin (Xa). To a suspension of 0.98 g (0.015 mole) of sodium azide in 10 ml of DMSO, we added over a 15 min period 3.36 g (0.01 mole) of compound Ia. We stirred the reaction mixture at room temperature for five hours and poured it into water (150 ml). We filtered off the precipitate, washed it with water, and dried it. On the basis of the corresponding 3-(bromacetyl)coumarins Ib, c [4], we prepared compounds Xb, c by a similar procedure.

3-(2-Triphenylphosphazoacetyl)-6,8-dichlorocoumarin (XIa). To a heated solution of 1.49 g (0.005 mole) of compound Xa in 20 ml of dichloroethane, we added a solution of 1.32 g (0.005 mol) of triphenylphosphine in 5 ml of dichloroethane. We boiled the mixture for one hour. After evaporation of the solvent, we ground the obtained oil with ether until crystallization. We prepared XIb,c in a similar way.

2-[6,8-Dichlorocoumarin-3-yl)-2-oxoethyl]-1-(*p*-nitrophenylazomethine)(XIIa). We boiled a mixture of 1.33 g (0.0025 mole) of compound XIa and 0.38 g (0.0025 mole) of *p*-nitrobenzaldehyde in 20 ml of toluene for one hour. We separated the precipitate and washed it with ether. We synthesized compounds XIIb, c in a similar manner.

3-(Thiocyanoacetyl)coumarin (XIIIb) and 3-(thiocyanoacetyl)-5,6-benzocoumarin (XIIIc). To a solution of 0.005 mole of the relevant compound Ib, c in 70 ml of methanol we added 0.40 g (0.005 mole) of sodium thiocyanate in 10 ml of methanol. We boiled the mixture for 1.5 h. We separated the precipitate and washed it with ether. IR spectrum of XIIIb, ν_{max}, cm^{-1}: 1610 (CN).

4-(Coumarin-3-yl)thiazole-2-one (XIVb). To a solution of 0.245 g (0.001 mole) of compound XIIIb in 7 ml of glacial AcOH, we added three drops of H_2SO_4. We boiled the reaction mixture for one hour, cooled it, and added 10 ml of water. We filtered the precipitate, washed it with water, and dried it.

1-(5,6-Benzocoumarin-3-yl)-3-phenylpropene-1-one (XVc). To a solution of 2.38 g (0.01 mole) of 3-acetyl-5,6-benzocoumarin in 20 ml of chloroform, we added 1.06 g (0.01 mole) of benzaldehyde and five drops of piperidine. We heated the mixture for five hours with simultaneous distillation of the chloroform-water azeotrope. We washed the residue with ether and filtered it off.

4-(5,6-Benzocoumarin-3-yl)-5,6-dihydro-6-phenyl-2(1H)-pyrimidinethione (XVIc). We boiled a mixture of 1 g (0.003 mole) of compound XVc, 0.45 g (0.006 mole) of thiourea, and 0.3 g of NaOH in 90 ml of ethanol with stirring for six hours. After cooling the solution, we filtered off the precipitate, washed it with water, and dried it.

BIOLOGICAL EXPERIMENTAL PART

The antimicrobial activity was determined by two-fold serial dilutions in a meat and peptone broth with pH 7.2 with respect to *E. coli* 12, *S. aureus* 209, *P. aeruginosa* 40, and *B. subtilis*. The minimum inhibiting concentrations of the substances for the fungi *C. albicans* and *S. cerevisiae* were determined by two-fold serial dilutions in Saburo's liquid medium with pH 5.6 [8]. A broad spectrum of antibacterial and antifungal activity was established the phosphorus- and nitrogen-containing derivatives of coumarins. The compounds IIIa, VIIIb, XIIIc, and XVIc stand out among these compounds in this respect (Table 2).

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