IMMUNOSUPPRESSIVE ACTIVITY OF THE SODIUM SALT OF ARYLIDENIMINOETHYL HYDROGEN SULFATE

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In continuation of our search for physiologically active compounds among aromatic aldehyde derivatives, we synthesized the sodium salts of some arylideniminoethane sulfonic acids. Certain hydroxyalkyloxybenzaldehydes and naphthaldehydes were used for condensation with sodium β-aminoethyl sulfate. The resultant compounds are water-soluble brightly colored powderlike substances with poor solubility in organic solvents (Table 1).

A comparison of the UV spectra of the compounds obtained clearly shows that the spectra of II, III, V, and VII have maximum absorption bands in the 370-420 nm region and that such absorption is absent in the spectra of I, IV, VI, and VIII. The appearance of longwave absorption in the spectra of hydroxyaldehyde alkylimines is a characteristic of these compounds which was studied and elucidated in [1, 2]. The intensity of this longwave band was shown to be dependent upon the structure of the starting aldehyde and the amine component. The alkylimines exhibited a greater intensity than did the arylamines. The transition from the benzene derivatives to the naphthalene derivatives was also accompanied by a greater intensity. Among the possible reasons for the emergence of that band are the intramolecular chelate type hydrogen bonds, the intermolecular hydrogen bonds with the polar solvents, the presence of a quinoid tautomer, and, finally, a $n \rightarrow \pi^*$ transition which is permissible in principle for these types of compounds. One can see from Table 1 that absorption is observed in the 370-420 nm region along with absorption at 215-235 nm (electron transfers in the aldehyde ring) and at 255-340 nm (electron transfers in the Ar—CH=N— system). The nature of this transfer was studied in several salicylidene- and 2-hydroxy-1-naphthylidenimines [2]. From the data cited in [2] it is apparent that the appearance of this longwave maximum is not related to the chemical-structural conversions of the compounds, but rather to the permissible transition of one of its forbidden transitions, i.e., the $n \rightarrow \pi^*$-transition.

It is known that one of the proofs favoring that relationship is the disappearance of the longwave band in an acid medium which we observed when the spectra of II, III, V, and VII were recorded in sulfuric acid. The fact that a longwave maximum exists in both the spectra of o-hydroxyalkylimines and the spectrum of p-hydroxyalkylimine (V) indicates that the intramolecular hydrogen bond is not responsible for its appearance.

Singlets of the exocyclic proton at 8.52, 9.02, and 8.92 ppm can be clearly seen in the PMR spectra of compounds II, VII, and VIII in DMSO-d_6, respectively. A comparison of the PMR spectra of compounds VII, VIII, and the condensation product of 2-hydroxy-1-naphthaldehyde to the monoethanolamines indicates the absence of the quinoid structure whose formation might have been assumed for the latter two compounds. The proton signal of the o-position hydroxyl group was observed in weak fields at 9.06-9.08 ppm and disappears upon the addition of D_2O.

Thus, all of the examined sodium salts of hydroxy- and alkyloxybenzylidene- and naphthalideniminoethyl hydrogen sulfate are characterized by a benzoid structure. The appearance of a longwave absorption maximum in the UV spectra of some of those acids is associated with the redistribution of the π-electron density of the entire system which leads to a permissible $n \rightarrow \pi^*$ transition.

EXPERIMENTAL CHEMICAL

PMR spectra were recorded on an XL Varian instrument (USA) with a working frequency

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TABLE 1. Physicochemical Properties of the Sodium Salt of Benzylidene- and Naphthylideniminoethyl Hydrogen Sulfate $RCH{=}NCH_2CH_2SO_3ONa$

Compound	R	mp, °C	R_f	UV spectrum, λ_{max}, nm (log ε)	Found N, %	Empirical formula	Calculated N, %	Yield, %
I	Ph	166—8	0,90	235 (3,8)	5,68	$C_9H_{10}NO_4NaS$	5,57	38,2
II	C_6H_4OH-2	196—8	0,66	215 (4,26), 255 (4,01), 320 (3,47), 395—410 (2,60)	5,42	$C_9H_{10}NO_5NaS$	5,24	95,0
III	$C_6H_3(OH)_2$-2,4	>350	0,83	220 (4,15), 305 (4,10), 370 (3,77)	5,02	$C_9H_{10}NO_6NaS$	4,94	44,0
IV	C_6H_4OMe-4	172—4	0,83	270 (4,20)	4,61	$C_{10}H_{12}NO_5NaS$	4,98	83,0
V	C_6H_3OMe-3-OH-4	232—4 (decomp.)	0,78	270 (4,07), 305 (3,93), 400 (3,87)	4,54	$C_{10}H_{12}NO_6NaS$	4,71	94,0
VI	$C_6H_3(OMe)_2$-3,4	203—5 (decomp.)	0,90	270 (4,14), 300 (3,89)	4,36	$C_{11}H_{14}NO_6NaS$	4,50	80,2
VII	$C_{10}H_6OH$-2	215—7 (decomp.)	0,58	235 (4,40), 300 (3,89), 400—420 (3,88)	4,35	$C_{13}H_{12}NO_5NaS$	4,41	88,0
VIII	$C_{10}H_6OMe$-2	178—80 (decomp.)	0,84	300 (3,92), 340 (3,56)	4,66	$C_{14}H_{14}NO_5NaS$	4,23	58,1

TABLE 2. Effect of the Sodium Salt of Arylideniminoethyl Hydrogen Sulfate on Antibody-Forming Cells in the Spleen of Mice Immunized with Sheep Blood

Compound	Dose, mg/kg	No. of antibody-forming cells per spleen		P
		average geometric number	95% confidence interval	
I	50	64 570	44 670—93 300	<0,001
	100	97 720	81 280—117 500	<0,001
	200	141 300	104 700—190 500	>0,5
II	50	75 860	53 700—107 200	<0,001
	100	79 430	67 610—93 300	<0,001
	200	85 110	70 790—102 300	<0,001
V	50	125 900	97 720—162 200	<0,5
	100	123 300	97 720—154 900	<0,2
	200	117 500	97 720—141 300	<0,5
VI	50	131 800	89 130—195 000	>0,5
	100	128 800	109 600—151 400	<0,5
	200	120 200	85 110—169 800	<0,5
VII	50	154 900	125 900—190 500	<0,2
	100	123 000	91 200—166 000	<0,5
	200	100 000	67 610—147 900	<0,05
Control		138 000	128 800—147 900	

Note. Five experimental mice; fifteen control mice.

TABLE 3. Effect of the Sodium Salt of Arylideniminoethyl Hydrogen Sulfate on the Survival Rate of Epidermal Allotransplants in Mice

Compound	Dose, mg/kg	Number of mice	Survival rate of allotransplants, days (M ± m)	P
I	50	12	13,0±0,54	<0,2
	100	12	14,2±0,28	<0,001
	200	10	15,5±0,38	<0,001
II	50	12	17,3±0,45	<0,002
	100	11	17,2±0,87	<0,001
	200	11	16,2±0,44	<0,001
V	50	10	14,9±0,72	<0,001
	100	10	15,0±0,47	<0,001
	200	10	15,6±0,77	<0,001
IV	50	12	10,5±0,5	<0,001
	100	10	16,4±0,48	<0,001
	200	11	17,5±0,93	<0,001
VII	50	12	16,1±0,48	<0,001
	100	11	16,7±1,06	<0,001
	200	9	20,0±0,07	<0,01
Control		36	12,0±0,49	

of 200 MHz. UV spectra were recorded on a SF-26 instrument in abs. methanol, c = 0.001%. Silufol UV-254 plates in a 1:20 hexane—methanol system were used for TLC.

Sodium Salt of 2-Methoxy-1-naphthalideneiminoethyl Hydrogen Sulfate (VIII). A 1.8-g (0.01 mole) portion of 2-methoxy-1-naphthaldehyde was added to a solution of 0.4 g (0.01 mole) of NaOH and 1.4 g (0.01 mole) of β-aminoethylsulfuric acid in 25 ml of abs. ethanol, heated to 60-70°C in a nitrogen stream. The reaction mixture was kept at 60-70°C for 2 h. After cooling the resultant precipitate was filtered off, washed with abs. ethanol, diethyl ether, and then dried. The yield and physicochemical constants are shown in Table 1. Compounds I-VII were obtained in a similar manner.

EXPERIMENTAL BIOLOGICAL

The accumulation of antibody-forming cells (AFC) in the spleens of mice immunized with sheep erythrocytes was used as an indicator of the effect that the test compounds had

on the humoral link of immunity [4]. The preparations were administered orally to the mice on the first, second, and third day after immunization. The degree to which AFC were suppressed in comparison to the control was indicative of the immunosuppressive activity of the test substances (Table 2).

The allotransplantation of a cutaneous flap [3] in BALB/c line mice (recipients) and CBA mice (donors) also served to identify the immunosuppressive activity of the compounds under study. At first, the animals were given the preparations orally one day prior to the transplantation, and then every other day until the allotransplant separated. The degree to which the viability of the cutaneous flap was prolonged in comparison to the control was used as an indicator of the substance's immunosuppressive activity (Table 3). Daily observations were made of the animals' general condition and behavior as well as of the transplant's condition.

The results showed that the compounds under study exhibit low toxicity. The LD_{50} for II was 120 mg/kg. The LD_{50} for the rest of the preparations could not be ascertained because no deaths among the animals were observed upon the administration of doses up to 200 mg/kg.

As can be seen from Tables 2 and 3, antibody formation was suppressed with statistical reliability by compounds I and II at all doses. Compounds V, VI, and VII had almost no effect on the accumulation of antibody producers in the spleens of sheep erythrocyte-immunized mice, with the exception of V and VII at a dose of 200 mg/kg.

Our study of the effect that preparations had on the cellular link of immunity (see Table 3), which plays a leading role in the separation of allotransplants, established that all of the test substances exhibit definite immunosuppressive activity which resulted in a prolongation of the cutaneous flaps' survival time in mice.

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