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To the 85th Anniversary of birthday of late Yu.G. Gololobov

Synthesis and Properties of Sulfo-Containing Tetrazolium Betaines and Their Formazan Precursors

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Abstract—Chromogenic redox indicators—3,5-diphenyl-2-(4-sulfophenyl)-2*H*-tetrazolium betaine and 2-(4-nitrophenyl)-5-phenyl-3-(4-sulfophenyl)-2*H*-tetrazolium betaine—were synthesized by the oxidative cyclization of 1,3-diphenyl-5-(4-sulfophenyl)formazan and 5-(4-nitrophenyl)-3-phenyl-1-(4-sulfophenyl)formazan under the action of N-bromosuccinimide. The synthesis of sulfoformazans is accompanied by formation of azo coupling products with substitution of the sulfo group in the hydrazone molecule by the arylazo group. Spectral and voltage–current characteristics of the synthesized compounds were studied.

Keywords: tetrazolium betaines, formazan, redox indicators

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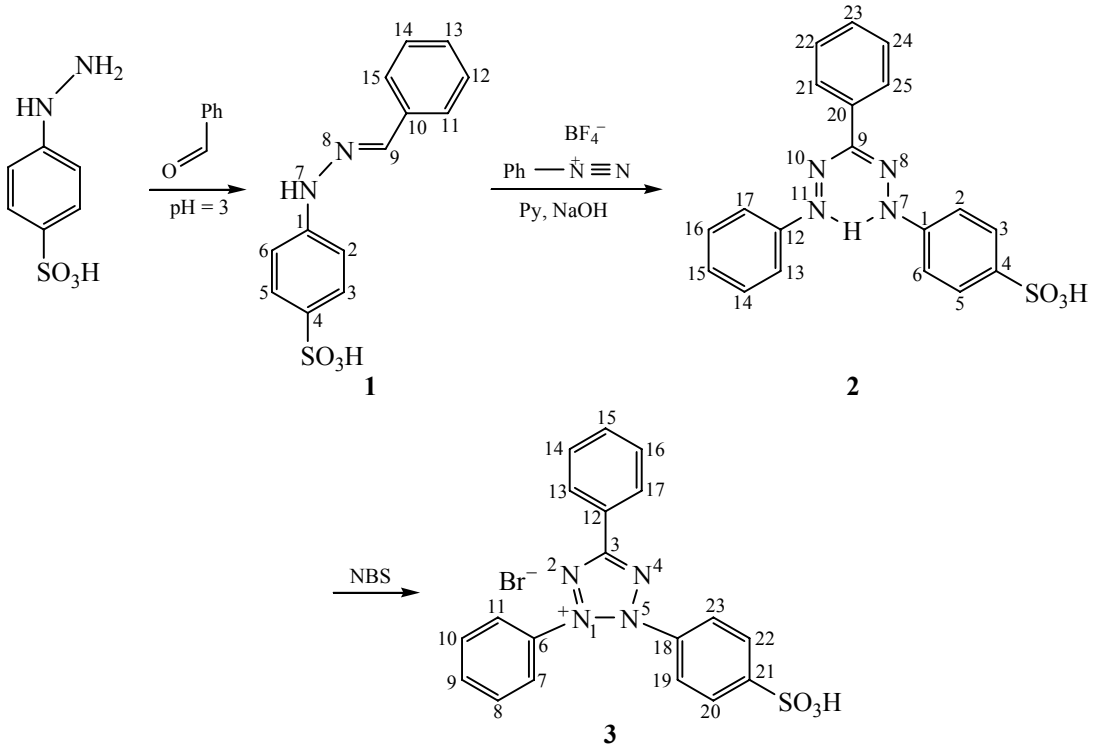
At present tetrazolium salts as electron acceptors have found diverse applications in biochemical, chemical analytical, and other research as chromogenic markers and redox indicators whose principle of action is based on the conversion of colorless redox indicators into brightly colored formazans. In particular, this property forms the basis for the use of tetrazolium salts for screening of anticancer drugs and assessment of the metabolic activity of live cells, and, therewith, special focus is on nitrotetrazolium salts which are highly sensitive to reduction under the action of dehydrogenases and apophorases [1–6].

It was found that tetrazolium salts are highly sensitive indicators of superoxide radicals [7, 8]. Useful applications of the chemical systems on the basis of tetrazolium salts in inorganic analysis [9] and preparative heterocyclic chemistry [10] have been described.

In the present work we set ourselves the goal to synthesize aryltetrazolium betaines by oxidative intramolecular cyclization of arylformazans containing, in one case, a sulfo-substituted phenyl ring and, in the other, nitro- and sulfo-substituted phenyl rings and compare the spectral and electrochemical characteristics of the synthesized compounds.

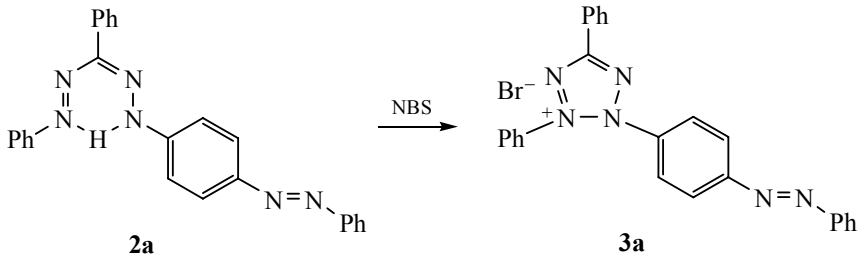
Tetrazolium salts are generally synthesized in two stages: the fierst involves synthesis of the corresponding formazans and the second, oxidation of the latter in various systems [11]. From the viewpoint of practical applications, of the greatest interest are water-soluble tetrazolium salts containing sulfo substituents. Fichter and Schiess described the synthesis of an inner tetrazolium salt, involving the synthesis of the sodium salt of 1,3-diphenyl-5-(4-sulfophenyl)formazan by the reaction of benzaldehyde (4-sulfophenyl)hydrazone with diazobenzene in aqueous sodium carbonate and subsequent oxidation of the synthesized salt with nitrous acid [12]. However, the authors of the cited work did not provide the procedure of the synthesis and any characteristics of the products (except for analyses for nitrogen and sulfur) to confirm their individuality and structure. The syntheses of a water-

Scheme 1.



1 **2** **3**

Scheme 2.



2a **3a**

soluble 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide [13] and a series of water-soluble tetrazolium salts containing SO_3, NO_2, COOH, OCH_3 substituents in the phenyl rings [14–16] were reported.

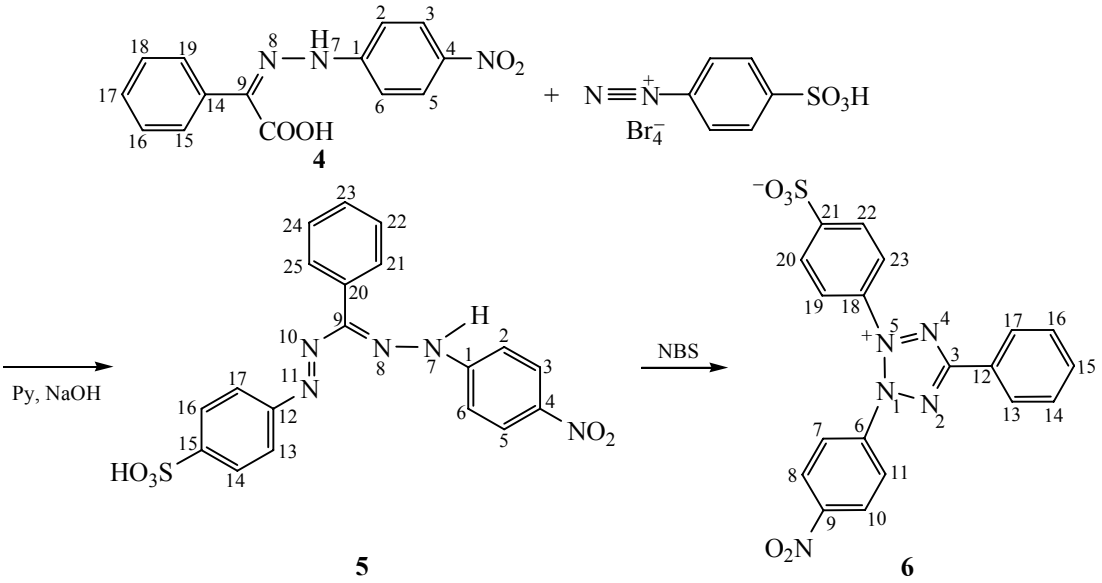
We synthesized two sulfo-containing tetrazolium betaines and their formazan precursors and studied their structure and properties by means of cyclic voltammetry, electronic absorption (EA), IR, and NMR spectroscopy, and mass spectrometry.

The synthesis of 3,5-diphenyl-2-(4-sulfophenyl)-2*H*-tetrazolium betaine (**3**) involved three stages: condensation of (4-hydrazinobenzene)sulfonic acid with benzaldehyde to obtain benzaldehyde (4-sulfo-phenyl) hydrazone (**1**), azo coupling of hydrazone **1** with diazobenzene to obtain 1,3-diphenyl-5-(4-sulfophenyl) formazan (**2**), and oxidation of the latter with *N*-bromosuccinimide (NBS) to betaine **3** (Scheme 1).

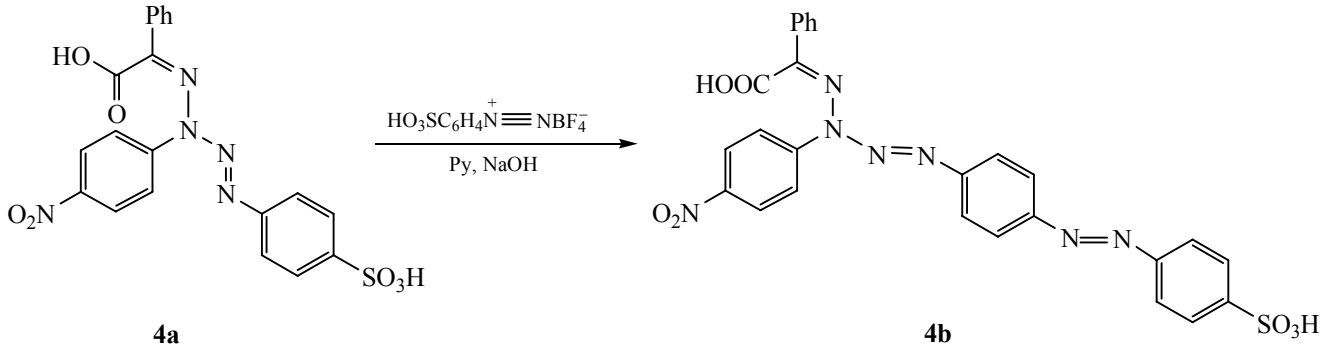
At the second stage we obtained, along with formazan **2**, two by-products formed by azo coupling of diazobenzene with the sulfo group and substitution of the sulfo group by phenylazo group: benzaldehyde [4-(4-phenylazo)-phenyl]hydrazone (**1a**) and 1,3-diphenyl-5-[4-(phenylazo)phenyl]formazan (**2a**), which were separated from the target product by extraction with chloroform. Formazan **2a** was oxidized with NBS to 3,5-diphenyl-2-(4-(phenylazo)phenyl)-2*H*-tetrazol-3-ium bromide (**3a**) (Scheme 2).

The synthesis of 2-(4-nitrophenyl)-5-phenyl-3-(4-sulfophenyl)-2*H*-tetrazolium betaine **6** involved two

Scheme 3.



Scheme 4.



stages: azo coupling of phenylglyoxalic acid (4-nitrophenyl)hydrazone (**4**) with 4-diazobenzenesulfonic acid to obtain 5-(4-nitrophenyl)-3-phenyl-1-(4-sulfophenyl)formazan (**5**) and oxidation of the latter to betaine **6** (Scheme 3).

The yield of fomazan **5** at the first stage involving substitution of the carboxylic group in hydrazone **4** by the arylazo group is low because of the occurrence of two side azo coupling reactions: substitution of the imino proton in hydrazone **4** under the action of 4-diazobenzenesulfonic acid by the 2-[2-(4-nitrophenyl)-4-(4-sulfophenyl)tetraazylidene]-2-phenylacetic acid (**4a**) and subsequent substitution of the sulfo group in tetrazene **4a** by the 4-sulfophenylazo group to form 2-[2-(4-nitrophenyl)-4-(4-sulfophenylazo)phenyltetraazylidene]-2-phenylacetic acid (**4b**) (Scheme 4).

Apparently, these reactions are associated with the deprotonation of the hydrazono NH group under the action of the electron-acceptor nitro group, as well as with the presence of still unsubstituted carboxylic group which prevents the tetrazene–formazan rearrangement.

Compound **4b** is insoluble in acetone, which allowed us to separate it from formazan **5** by extraction of the latter with acetone. Formazan **5** was obtained in a low yield, and, therefore, it was synthesized with a higher yield by azo coupling of benzaldehyde (4-nitrophenyl)hydrazone with 4-diazobenzenesulfonic acid.

We studied the spectral characteristics of compounds **1**–**6**.

Tetrazolium betaines **3** and **6** are insoluble in absolute ethanol and soluble in aqueous ethanol; the

Table 1. Parameters of the EA spectra of tetrazolium betaines **3** and **4** and typical tetrazolium salts

Compound	λ, nm			ε_{max}, L mol^{-1}cm^{-1}		
	λ_{max1}	λ_{min}	λ_{max2}	$\varepsilon_{max1} \times 10^4$	$\varepsilon_{min} \times 10^4$	$\varepsilon_{max2} \times 10^4$
3	200	224	252	3.64	1.04	1.91
4	203	226	260	3.92	1.03	2.60
2,3,5-Triphenyl-2*H*-tetrazolium chloride	201	220	250	3.64	0.67	1.64
2-(4-Nitrophenyl)-3,5-diphenyl-2*H*-tetrazolium chloride	203	225	259	3.70	1.08	2.27

solution of betaine **3** is colorless, like the solution of 2,3,5-triphenyl-2*H*-tetrazolium chloride, while the solution of betaine **6** has a light yellow color, like that of 2-(4-nitrophenyl)-3,5-diphenyl-2*H*-tetrazolium chloride. As seen from Table 1, the EA spectra of the mentioned two couples of compounds are similar to each other, but the bands in the spectra of nitrophenyl derivatives are slightly shifted red and have higher extinction coefficients ε.

In the ^{1}H NMR spectrum of compound **1,** the *p*-$C_6H_4SO_3H$ protons appear as an AX system. The observation in the NOESY spectrum of cross-peaks between the C^7H and C^9H protons suggests their spatial proximity and, consequently, trans configuration of the enimine fragment. Note that the NOESY spectrum also shows correlations between the NH and C^2H и C^6H *ortho*-proton signals, as well as between the C^9H and $C^{11}H$ и $C^{15}H$ *ortho*-proton signals, which provides evidence for correct assignment of the ^{1}H NMR spectrum of compound **1**.

The ^{1}H NMR spectrum of compound **2** contains an AX system from the *p*-$C_6H_4SO_3H$ protons, as well as characteristic signals of two unsubstituted phenyl rings. The NH proton signals is observed at 14.17 ppm, which suggests strong H-bonding in the 6-membered heteroring. The assignment of the δ_C signals of two different phenyl rings was based on the HMBC correlations between the C^{12} and C^{20} signals at 148.83 and 136.25 ppm and the triplet proton signals at 7.54 and 7.48 ppm, respectively, as well as the cross-peak between the C^9H signal and the doublet signal of the $C^{21,25}H$ proton at 7.99 ppm

Some signals in the NMR spectra of compound **3** were assigned using 2D HMQC and HMBC correlation techniques, as well as the ^{13}C NMR spectra registered in the JMODECHO mode. In view of the fact that the aromatic proton signals at 7.7−7.9 ppm were overlapping multiplets, the NMR spectra of this compound could not be assigned completely. We can only believe that we have strong grounds to assign the multiplet at 8.35 ppm to the $C^{13,17}H$ proton, which shows a double-intensity signal at 130.70 ppm in the HMQC spectrum. This conclusion is based on the observation in the HMBC spectrum of a cross-peak between this multiplet and the signal at 164.6 ppm, associated without any doubt with C^3. The latter, in its turn, gives a correlation signal with the C^{15} signal (δ_C = 134.10 ppm) from among other surrounding CH-signals. This signal can be attributed to *para*-carbon atoms in two phenyl rings. Thus, the second downfield signal at 134.71 ppm is assignable to C^9.

The ^{1}H NMR spectrum of compound **4** shows an AX system from the *p*-$C_6H_4NO_2$ protons at 7.48 ($C^{2,6}H$) and 8.19 ppm ($C^{3,5}H$). The NH proton signal is observed at 11.83 ppm, implying a lack of the potentially possible NH···O=C hydrogen bond.

The ^{1}H NMR spectrum of compound **5** contains signals of two AX systems characteristic of *para*-substituted benzene rings. According to the COSY spectrum, the doublet at 8.25 ppm is associated with the doublet at 7.69 ppm, whereas the doublet at 8.10 ppm is associated with the doublet at 7.83 ppm, and, therewith, the *J* constant for the first AX system (9.2 Hz) is much larger than for the second (6.8 Hz). In the ^{1}H NMR spectrum, the unsubstituted phenyl group appears as a doublet from *ortho* protons at 7.79 ppm (*J* = 7.0 Hz) and two overlapping triplets of the meta and para protons at 7.44−7.49 ppm. The NH proton gives a downfield signal (12.69 ppm), implying that com-pound **5** contains a 6-membered ring with an intra-molecular hydrogen bond between this proton and the N^1 atom. The presence in the spectrum of a correlation signal between the NH proton and the signal at δ_C = 114.85 ppm, which, according to the

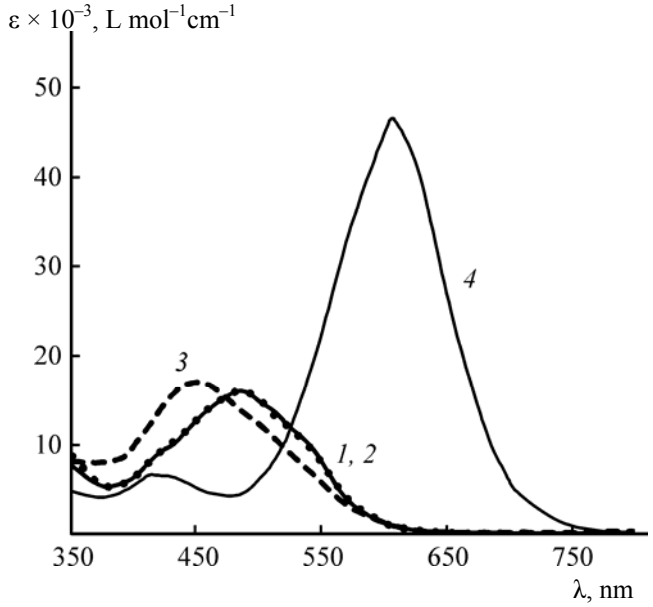


Fig. 1. Electronic absorption spectra of (*1*, *2*) formazan **2** and (*3*, *4*) formazan **5** at (*1*, *3*) pH 6 and (*2*, *4*) 12.

HMQC spectrum, corresponds to the proton signal at 7.69 ppm, shows that the AX spin system at 7.69 and 8.25 ppm belongs to the *p*-$C_6H_4NO_2$ substituent.

The ^{1}H NMR spectrum of compound **6** shows signals of two AX systems from two *para*-substituted phenyl rings. The first system (8.16 ppm, $C^{7,11}H$; 8.58 ppm, $C^{8,10}H$, *J* 9.2 Hz) belongs to the *p*-$C_6H_4NO_2$ system. One component of the second AX system of the *p*-$C_6H_4SO_3H$ substituent is observed at 7.90 ppm ($C^{20,22}H$, *J* = 8.6 Hz), and the other component of this system ($C^{19,23}H$) overlaps with the multiplet ($C^{14,15,16}H$) from the unsubstituted phenyl ring at 7.77−7.85 ppm. The doublet of the *ortho* protons $C^{13,17}H$ appears at 8.36 ppm.

Table 2. Comparative characteristics of formazans **2** and **5** and crown formazans **7**−**10**

Comp. no.	Conformer	δ_H(NH), ppm	λ_{max}, nm
2	*EZZ*	14.17	482
5	*EEZ*	12.69	450
7	*EZZ*	14.21	552
8	*EZZ*	14.00	495
9	*EEZ*	11.56	480
10	*EEZ*	12.18	454

In the ^{1}H NMR spectra of compounds **1**–**6,** larger downfield shifts are characteristic of all the nitro derivatives, as well as in the tetrazolium cation compared to its formazan precursor. Signals of the aryl substituent at the azo group are observed exclusively in formazans and tetrazolium betaines.

Solid formazans **2** and **5** exist in a chelate *EZZ* conformation with intramolecular hydrogen bonds. At the same time, the tautomeric equilibria of these compounds in solutions differ from each other Aqueous ethanolic solutions of formazans **2** and **5** are colored red and orange-red, respectively. The EA maximum of nitroformazan **5** which has a weaker intramolecular hydrogen bond is shifter blue with respect to the EA maximum of formazan **2** (Fig. 1), even though the nitro group usually imparts a more intense color.

As known, the NH proton in an *EZZ* chelate arylformazan alternately forms a covalent bond with N^1 or N^5 due to intrachelate proton transfer (the lifetime of the tautomers is about 10^{-3} s). Based on the $^2J(^{13}CNH)$ values we showed the introduction of the strongly acceptor NO_2 group into one N-aryl ring in 2,3,5-triphenyl-2*H*-tetrazolium chloride almost completely shifts the tautomeric equilibrium to 4-$NO_2C_6H_4NH$ [17, pp. 29−32]. Therewith, the δ_H(NH) decreases by 2 ppm. As the formazan is present as two conformers (*EZZ* and and *EEZ*), this proton chemical shift is an average value due to fast (on the NMR scale) prototropic tautomeric interconversions in the formazan chain.

In an alkaline medium (pH = 12), the absorption maximum of formazan **2** remains remains almost unchanged, because the strong intramolecular bond is not broken. By contrast, an alkaline solution of formazan **5** acquires a dark blue color, and its absorption maximum shifts red by 163 nm, and its ε becomes markedly higher (Fig. 1). This result is explain by the fact that the electron-acceptor nitro group causes breakage of the intramolecular hydrogen bond, deprotonation of the $NO_2C_6H_4NH$ group, and rearrangement of the chromophoric system of the molecule.

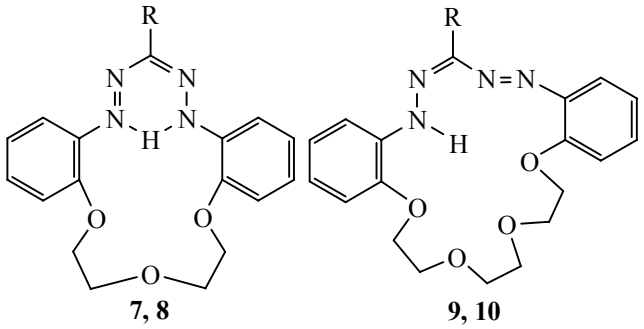
Comparison of the δ_H(NH) and λ_{max} values for **2** and **5** with the respective values for crown formazans **7**−**10** which exist as a single conformer having the formazan fragment is rigidly incorporated into the ring [18, 19] (Table 2) provides further evidence for the

Table 3. Parameters of the IR spectra (ν, cm^{-1}) of compounds **1–6**

Assignment	1	2	3	4	5	6
N–H	3320	–	–	3280	–	–
$O–H_{sulfo}$	3070	3060	3064	–	3080	3070
$C=O_{COOH}$	–	–	–	1680 s	–	–
$C=C_{Ar}$	1608 s 1596 s 1568 m	1644 w, br 1596 s 1448 m	1608	1600 s 1580 m 1560 m	1596 s 1444 m	1584 m
C=N	1524 s	1512 s	1528	1536 s	1516 s. ш	1536 s
$C–C_{Ar}$	1492 m	1492 s	1488	1504	1516	1488 m
N=N	–	1456 s	1456 s	–	1456 s, sh	1456 s
$\nu_s(C–N)$	1356 w	1356 m	1344 w	1332 s, br	1344 s	1348 s
$\nu_s(C–NO_2)$	–	–	–	1300 m	1300 w	1307 sh
N–N	1290 m	1228 s	1232 s	1240 s, br	1280 s	1288 m
SO_3H	1260 s 1192 vs 1036 s	1210 sh 1184 s 1040 s	1208 s 1180 s, sh 1036 s	–	1230 s, br 1188 s 1108 s	1232 s 1212 s 1108 m
$C–C_{disubst}$	1124 vs	1124 vs	1116 s	1112 s	1124 s	1180 s
$–C–C_{hydrazone}$	932 m	984 m	996 m	992 m	1008 m	1008 m
$\gamma(CH_{monosubst})$	760 m 696 m	760 s 688 m	772 m 692 m	792 m 688 m	752 m 696 m	764 m 692 m
$\gamma(CH_{Ar})$	636 m	640 m	640 s	–	620 m	644 m

EZZ configuration of formazan **2** and *EEZ* configuration of formazan **5**.

Comparison of the IR spectra of hydrazones, formazanes, and tetrazolium salts **1–6** (Table 3) confirms the molecular structures and the presence in them of azo, sulfo, or nitro groups. Characteristic ν (NH) bands are observed only in the spectra of hydrazones, which suggests that solid formazans **2** and **5** have a stable chelate structure.



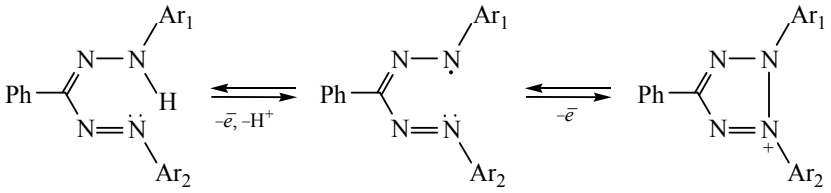
7, 8 **9, 10**

R = Ph (**7**), CN (**8**, **9**), H (**10**).

Electrochemical characteristics of compounds 2, 3, 5, and 6. The electrochemistry of the tetrazolium salt–formazan system is fairly complicated, because the electrode process involves many stages and certain intermediate products are unstable [20–25]. Figure 2 shows the cyclic voltammograms of the aqueous alcoholic solutions of compounds **2**, **3**, **5,** and **6**, measured on a glassy-carbon electrode. As seen, in the range from –1.4 V to +1.0 V formazans **2** and **5** undergo quasiequilibrium oxidation (Fig. 2a), whereas betaines **3** and **6** under go quasiequilibrium reduction (Fig. 2b). Therewith, all curves contain two pairs of redox peaks, implying two-stage electrode processes which are likely to involve intermediate formation of tetrazolyl radicals. In view of the data in [25], the electrooxidation of formazans can be presented by the Scheme 5.

The cathodic and anodic peak potentials for the synthesized formazans **2** and **4** are listed in Table 4. As seen from the data in the table, the anodic peak potentials which relate to the tendency of these

Scheme 5.



compounds for oxidation are almost the same, whereas the cathodic peak potentials measured on the reverse potential sweep much differ from each other. Therewith, it is obvious that the introduction of the nitro group into sulfo-substituted formazan **2** results in an appreciable cathodic shift of the electroreduction potential. The difference of the formal redox pontials in this case is about 50 mV. These data correlate with the characteristic electrochemical parameters of the synthesized tetrazolium betaines **3** and **6** and unsubstituted 2,3,5-triphenyl-2*H*-tetrazolium chloride (Table 5). All other factors being equal, the peak currents for nitrophenyl derivatives are much higher than for the other compounds.

Reductive properties of compounds 3 and 6. The photometric study of the chemical reduction of the synthesized sulfo-containing tetrazolium betaines to the corresponding formazans in solutions under the action of sodium sufide revealed considerable differences in the reaction kinetcs (Fig. 3). Thus, compound **3** was reduced within 25 min, while compound **6**, within 2 min.

EXPERIMENTAL

The electronic absorption (EA) spectra of formazans and tetrazolium betaines (5×10^{-5} M) were registered on a Jenway spectrophotomer (Bibby Scientific, England), solvent ethanol–water (1 : 1). The ^{1}H and ^{13}C NMR spectra were measured on a BrukerAvance^{TM}600 spectrometer at 600.22 and 150.93M Hz, respectively; solvent DMSO-d_6, internal reference TMS. The mass spectra (MALDI) were obtained on a Bruker Ultraflex instrument. The IR spectra in the range 3800–400 cm^{-1} were recorded on a Specord M in KBr. Elemental analysis was performed on a Carlo Erba instrument. Cyclic voltammograms were registered on an Ekotest-VA (Ekoniks-Ekspert, Moscow) with a glassy carbon working electrode, a

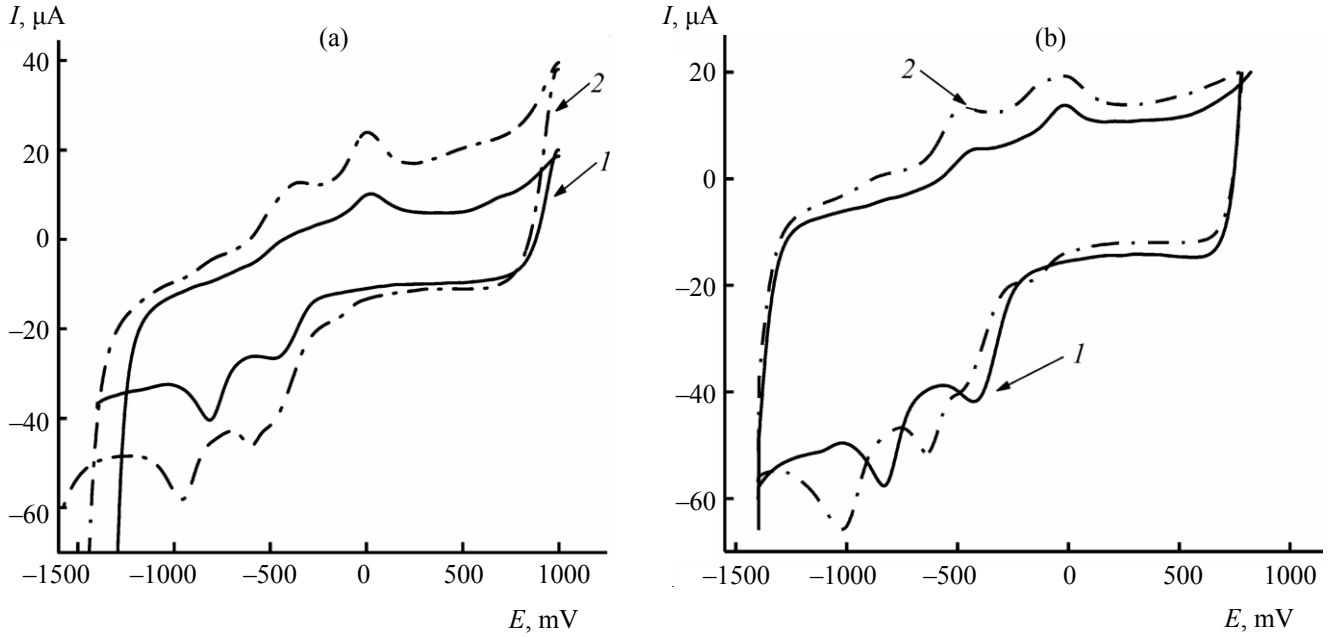


Fig. 2. Cyclic voltammograms of formazans (a, *1*) **2** and (a, *2*) **5** and tetrazolium betaines (b, *1*) **3** and (b, *2*) **6**.

Table 4. Voltage–current characteristics of formazans **2** and **5**

Comp. no.	Oxidation peak potential, V		Reduction peak potential, V		Formal redox potential, V	
	E^1_{pa}	E^2_{pa}	E^2_{pc}	E^1_{pc}	$E^{0'}_1$	$E^{0'}_2$
2	–0.348	0.008	–0.437	–0.826	–0.597	–0.259
5	–0.350	0.011	–0.602	–0.984	–0.667	–0.295

silver–silver chloride electrode (Ag/AgCl, KCl_{sat}) reference electrode, and platinum auxiliary electrode. Cyclic chronovoltammograms were registered in 40% aqueous ethanolic solutions (0.15 M NaCl) at the polarization potential sweep rate 0.1 V/s. The optical density of the solutions in the kinetic study of the reduction of tetrazolium betaines (3×10^{-4} M) in aqueous ethanol (1 : 1) with 1% sodium sulfide was measured in quartz cells (10 mm) on an Ekspert-003 miniphotometer (Ekoniks-Ekspert, Moscow) at 524 nm.

Benzaldehyde (4-sulfophenyl)hydrazone (1). A mixture of 18.8 g (0.1mol) of (4-hydrazinobenzene) sulfonic acid, 40 mL of water, 10 mL of acetic acid, and 11.7 g (0.1 mol) of benzaldehyde was stirred at 60°C for 3 h. The reaction product was precipitated with saturated aqueous sodium chloride and twice recrystallized from 70% ethanol. Yield 19 g (72%), mp 243−246°C (decomp.). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.01 d (2H, $C^{2,6}H$, *J* 8.6), 7.30 t (H, $C^{13}H$, *J* 7.4 Hz), 7.39 t (2H, $C^{12,14}H$, *J* 7.6 Hz), 7.50 d (2H, $C^{3,5}H$, *J* 8.6), 7.65 d (2H, $C^{11,15}H$, *J* 7.3), 7.88(1H, C^9), 10.49 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 111.10 ($C^{2,6}$), 126.18 ($C^{11,15}$), 127.31 ($C^{3,5}$), 128.53 (C^{13}), 129.14 ($C^{12,14}$), 136.13 (C^{10}), 137.56 (C^9), 139.11 (C^4), 145.85 (C^1). Mass spectrum, *m*/*z* (I_{rel}, %): 196 (16) $[M - SO_3]^+$, 178 (100) $[M - SO_3 - NH_3]^+$. M_{calc} 31. Found, %: C 49.38; H 4.87; N 8.45; S 10.42. $C_{13}H_{12}N_2O_3S \cdot 2H_2O$. Calculated, %: C 49.99; H 5.16; N 8.97; S 10.27. At 80°C and 10 mmHg the compound loses 11.2% of weight, which corresponds to the fraction of the water of crystallization. Found, %: C 56.11; H 4.58; N 9.85; S 11.13. $C_{13}H_{12}N_2O_3S$. Calculated, %: C 56.51; H 4.38; N 10.14; S 11.60. The IR spectral data are listed in Table 3.

1,3-Diphenyl-5-(4-sulfophenyl)formazan (2). A mixture of 1.91 g (15 mmol) of aniline hydrochloride, 12 mL of water, 3 mL of conc. HCl, 1.7 g (15 mmol) of sodium tetrafluoroborate, and 10.4 mL of 10% sodium nitrite (15 mmol) was stirred at 0−2°C for 1 h and then added in portions to a solution of 4.14 g (15 mmol) of hydrazine **1** in 40 mL of 6% sodium hydroxide. After 3-h stirring at 1−3°C the pH of the reaction mixture was adjusted to 2 with HCl. The precipitate that formed was treated with chloroform and methylene chloride to remove compounds containing no sulfo group. The reaction product was extracted with a methanol–acetone mixture (1 : 1) and reprecipitated with diethyl ether from methanol. Yield 0.8 g (14%), dark red powder, mp 195−197°C (decomp.). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.38 t (2H, $C^{14,16}H$, *J* 4.6), 7.40 t (2H, $C^{22,24}H$, *J* 4.6), 7.48 t (1H, $C^{23}H$, *J* 5.0), 7.54 t (1H, $C^{15}H$, *J* 5.1), 7.70 d (2H, $C^{2,6}H$, *J* 5.5), 7.77 d (2H, $C^{13,17}H$, *J* 5.7), 7.91 d (2H,

Table 5. Characteristic electroreduction parameters of the synthesized tetrazolium betaines and 2,3,5-triphenyl-2*H*-tetrazolium chloride

Compound	Reductionpeak potential, V		Formal redox potential, V	
	E^2_{pc}	E^1_{pc}	$E^{0'}_1$	$E^{0'}_2$
2,3,5-Triphenyl-2*H*-tetrazolium chloride	–0.438	–0.873	–0.428	–0.868
3	–0.442	–0.835		
6	–0.640	–1.017		

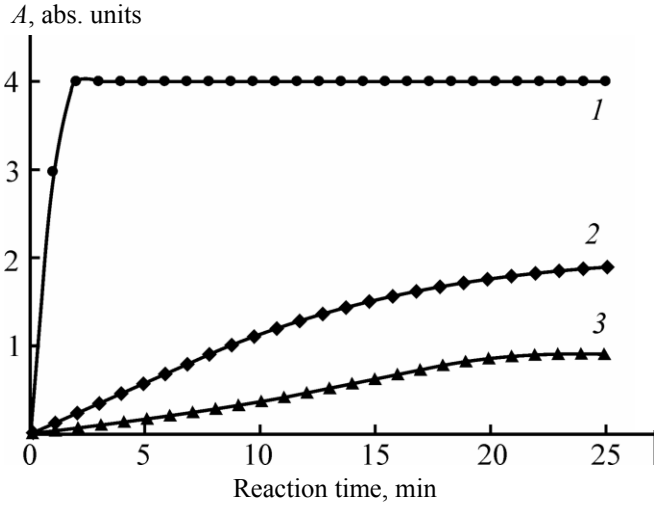


Fig. 3. Plots of optical density against time for solutions of tetrazolium salts (*1*) **6**, (*2*) **3,** and (*3*) 2,3,5-triphenyl-2*H*-tetrazolium chloride in the course of reduction with sodium sulfide.

$C^{21,25}H$, *J* 5.2), 7.99 d (2H, $C^{3,5}H$, *J* 5.2), 14.17 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 118.07 ($C^{2,6}$), 120.14 ($C^{13,17}$), 126.88 ($C^{21,25}$), 127.30 ($C^{3,5}$), 128.40, 128.81 ($C^{15,23}$), 128.93 ($C^{22,24}$); 129.97 ($C^{14,16}$), 136.25 (C^{20}), 142.51 (C^9), 146.98 (C^4), 147.31 (C^1), 148.83 (C^{12}). Mass spectrum, *m*/*z* (I_{rel}, %): 380.36 (70) $[M]^+$, 301 (100) $[M - SO_3]^+$, 457 (48) $[M + Ph]^+$. M_{calc} 380.43. Found, %: C 58.63; H 4.35; N 14.36; S 7.99. $C_{19}H_{16}N_4SO_3$. Calculated, %: C.99; H 4.24; N 14.73; S 8.43. The IR spectral data are listed in Table 3.

The extract was evaporated to leave a dark brown powder. Thin-layer chromatography of the latter gave **benzaldehyde 4-[(4-phenylazo)phenyl]hydrazone (1a)** (R_f 0.72, *m*/*z* 300.36 $[M]^+$, M_{calc} 300.81), which was previously obtained by azo coupling of benzaldehyde phenylhydrazone with diazobenzene at pH 3 (mp 167–169°C [26]), and **1,3-diphenyl-5-(4-(phenylazo)phenyl)-formazan (2a)** (R_f 0.21, mp 185°C, *m*/*z*: 404.60 $[M]^+$, M_{calc} 404.44), which was previously obtained by azo coupling of benzaldehyde phenylhydrazone with 4-diazobenzene (mp 182°C [27]). Formazan **2a** was treated with *N*-bromosuccinimide to obtain

3,5-diphenyl-2-(4-(phenylazo)phenyl)-2*H*-tetrazol-3-ium bromide (3a). Mass spectrum, *m*/*z*: 483.20 $[M]^+$, 403.37 $[M - Br]^+$. M_{calc} 483.25.

3,5-Diphenyl-2-(4-sulfophenyl)-2*H*-tetrazolium betaine 3. A mixture of 0.38 g (1 mmol) of formazan **2** in 15 mL of ethanol and 0.32 g (2 mmol) of *N*-bromosuccinimide until the reaction mixture changed color to light yellow. The solution was filtered off, and the product was precipitated with diethyl ether and recrystallized from 90% ethanol, washed with boiling absolute ethanol, and dried to obtain an off-white powder which did not melt up to 265°C. Yield 0.18 g (47%). ^1H NMR spectrum, δ, ppm (*J*, Hz): 7.73–7.88 m (12H, $C^{7,8,10,11,14–16,19–23}H$), 8.35 d (2H, $C^{13,17}H$, *J* 7.3). ^{13}C NMR spectrum, δ_C, ppm: 123.36 (C^6), 126.67 (2CH), 126.80 (2CH), 127.77 (2CH), 127.89 (2CH), 130.70($C^{13,17}$), 130.99 (2CH), 133.05 (C^{12}), 133.42 (C^{18}), 134.10 (C^{15}), 134.71 (C^9), 153.50 (C^{21}), 164.51 (C^3). The EA and IR spectral data are listed in Tables 1 and 3, respectively.

Phenylglyoxalic acid (4-nitrophenyl)hydrazone (4) was synthesized by the procedure in [28]. Yield 62%, mp 162−163°C. ^1H NMR spectrum, δ, ppm (*J*, Hz): 7.42 t (2H, $C^{16,18}$, *J* 4.7), 7.45 d (2H, $C^{15,19}H$, *J* 6.9), 7.48 d (2H, $C^{2,6}H$, *J* 9.2), 7.71 t (1H, $C^{17}H$, *J* 8.4), 8.19 d (2H, $C^{3,5}H$, *J* 9.3), 10.40 s (1H, NH), 11.85 s (1H, COOH). ^{13}C NMR spectrum, δ_C, ppm: 113.79 ($C^{2,6}$), 126,24 ($C^{3,5}$), 127.95 ($C^{15,19}$), 128.74 ($C^{16,18}$), 129.13 (C^{17}), 135.47 (C^{14}), 137.27 (C^9), 140.85 (C^4), 150.32 (C^1), 164.88 (C^{10}). Mass spectrum, *m*/*z* (I_{rel}, %): 285 (100) $[M]^+$, 241 (70) $[M - CO_2]^+$, 178 (100) $[M - CO_2 - NO_2 - NH_3]^+$. M_{calc} 285.26. Found, %: C 55.12; H 4.25; N 13.17. $C_{14}H_{11}N_3O_4$ H_2O. Calculated, %: C 55.45; H 4.32; N 13.86. At 80°C and 10 mmHg the compound loses 5.2% of weight, which corresponds to the fraction of the water of crystallization. Found, %: C 58.73; H 3.66; N 14.81. $C_{14}H_{11}N_3O_4$. Calculated, %: C 58.95; H 3.87; N 14.73. The IR spectral data are listed in Table 3.

5-(4-Nitrophenyl)-3-phenyl-1-(4-sulfophenyl)formazan (5). A mixture of 0.87 g (5 mmol) of sulfanylic acid, 9 mL of water, 1.4 mL of conc. HCl, 0.88 g (5 mmol) of 4-toluenesulfonic acid, and 3.5 mL of 10% sodium nitrite (5 mmol) was stirred at –1°C for 1 h, and then added in portions to a stirred suspension of 1.21 g (5 mmol) of benzaldehyde 4-nitrophenylhydrazone in 75 mL of DMF, 15 mL of pyridine, and 2 g of sodium carbonate. After 2-h stirring at 1−3°C, the reaction product was precipitated with conc. HCl, washed with water and isopropanol, extracted with acetone, and reprecipitated with diethyl ether from methanol. Yield 0.32 g (15%), dark red powder, mp 220−222°C. ^1H NMR spectrum, δ, ppm (*J*, Hz): 7.44−7.46 m (3H, $C^{22,23,24}$), 7.69 d (2H $C^{2,6}H$, *J* 9.2) 7.79 d (2H, $C^{21,25}H$, *J* 7.0), 7.83 d (2H, $C^{13,17}H$, *J*.8), 8.10 d (2H, $C^{3,5}H$, *J* 6.8), 8.25 d (2H, $C^{14,16}H$, *J* 9.2), 12.69 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 114.85 ($C^{2,6}$), 123.69 ($C^{14,16}$), 126.22 ($C^{3,5}$), 127.14 ($C^{13,17}$), 128.69 ($C^{21,25}$), 128.76 (C^{23}), 129.33 ($C^{22,24}$), 133.80 (C^{20}), 141.30(C^4), 147.71 (C^9), 149.98 (C^1), 152.32 (C^{12}), 152.40 (C^{15}). Mass spectrum, *m*/*z* (I_{rel}, %): 425 (100) $[M]^+$, 317 (100) $[M - SO_3 - N_2]^+$, 178 (100) $[M - SO_3 - N_2 - Ph - NO_2 - NH_3]^+$. M_{calc} 425.43. Found,%: C 54.04; H 3.40; N 16.28; S. 7.34. $C_{19}H_{15}N_5O_5S$. Calculated, %: C 53.65; H 3.53; N 16.45; S 7.52. The IR spectral data are listed in Table 3.

Formazan **5** was also prepared by azo coupling of 4-diazobenzenesulfonic acid with hydrazone **4.** However, the yield of the target product in this synthesis was as little as 3%; the main reaction products were 2-[2-(4-nitrophenyl)-4-(4-sulfophenyl)tetraazylidene]-2-phenylacetic acid (**4a**) and 2-([-(4-nitrophenyl)-4-(4-sulfophenylazo)phenyltetraazylidene]-2-phenylacetic acid (**4b**) formed by substitution of the sulfo group in tetrazene **4a** by the 4-sulfophenylazo group. Mass spectrum, *m*/*z* (I_{rel}, %): **4a**, 469.20, M_{calc} 469.44; **4b**, 574.30, M_{calc} 573.55.

2-(4-Nitrophenyl)-5-phenyl-3-(4-sulfophenyl)-2*H*-tetrazolium betaine (6) was prepared similarly to tetrazolium betaine **3** from 0.4 mmol of formazan **5** and 0.8 mmol of *N*-bromosuccinimide. Yield 0.12 g (70%), light yellow powder, doe not met up to 265°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.77–7.85 m (5H^{14,15,16,19,23}), 7.90 (2H, C^{20,22}H, *J* 8.6), 8.16 d (2H, C^{7,11}H, *J* 9.2), 8.36 d (2H, C^{13,17}H, *J* 8.6), 8.58 d (2H, C^{8,10}H, *J* 9.2). ^{13}C NMR spectrum, δ_C, ppm: 123.04 (C^{12}), 126.46 (C^{13,17}), 126.68 (C^{19,23}), 127.84 (C^{13,17}), 128.16 (C^{20,22}), 128.78(C^{7,11}), 130.76 (C^{14,16}), 132.75 (C^{18}), 134.33 (C^{15}), 137.22 (C^{6}), 150.87 (C^{9}), 153.73 (C^{21}), 165.97 (C^{3}). Mass spectrum, *m*/*z* (I_{rel}, %): 423 (95) $[M]^+$, 317 (100) $[M - SO_3]$, M_{calc} 423.41. Found, %: C 53.37; H 2.91; N 16.21; S 7.18. $C_{19}H_{13}N_5O_5S$. Calculated, %: C 53.96; H 3.09; N 16.54; S 7.57. The EA and IR spectral data are listed in Tables 1 and 3, respectively.

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