TETRAZOLIUM SALTS (REVIEW)

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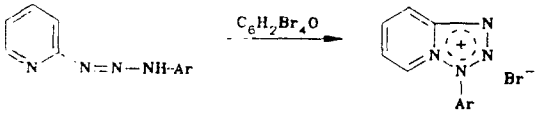
A summary is given for the methods of preparing tetrazolium salts, the physicochemical properties of these compounds, and their application in research and industry.

1,2,5-, 1,3,5-, 1,4,5-, and 2,3,5-Trisubstituted tetrazolium salts have been reported. In the present review, mesoionic salts, which are internal salts containing a positively charged tetrazolium ring, are also considered to be tetrazolium salts. 2,3,5-Trisubstituted tetrazolium salts were first obtained in 1894 by the oxidation of 1,3,5-triarylformazanes [1]. Information on 1,2,5-, 1,3,5-, and 1,4,5-trisubstituted tetrazolium salts appeared only much more recently when Messmer and Gelleri [2] reported the formation of 1,2,5-trisubstituted tetrazolium salts upon the oxidation of the corresponding 1,3-disubstituted triazenes [2] and Benson et al. [3] obtained 1,3,5- and 1,4,5-trisubstituted derivatives upon the alkylation of 1,5- and 2,5-disubstituted tetrazoles. Almost no attention was given to these compounds for almost 50 years after their discovery. This situation began to alter markedly at the end of the 1940s when Smith [4] found that, in alkaline medium, 2,3,5-triaryltetrazolium salts oxidize aldoses, ketoses, and other α-ketols and are reduced to brightly colored, water-insoluble formazanes [4]. Subsequently, the number of publications devoted to these compounds has steadily been increasing. A significant portion of these publications includes patents on the use of tetrazolium salts in science and industry. The data on tetrazolium salts published up to 1969 have been examined in reviews by Nineham [5] and Hooper [6]. Our review mainly concerns results obtained in the past decade.

METHODS OF PREPARATION

The set of methods for the synthesis of tetrazolium salts is rather limited. 1,2,5-Trisubstituted tetrazolium salts may be obtained upon the oxidation of 1,3-disubstituted triazenes, while 1,3,5- and 1,4,5-trisubstituted derivatives may be obtained by the alkylation of 1,5- and 2,5-disubstituted tetrazoles or by the reaction of nitrilium salts with alkyl or aryl azides. In the latter case, 1,4,5-trisubstituted tetrazolium salts are formed. The major method for the preparation of 2,3,5-trisubstituted tetrazolium salts involves the oxidation of 1,3,5-trisubstituted formazanes.

1,2,5-Trisubstituted Tetrazolium Salts. 1,2,5-Trisubstituted tetrazolium salts have hardly been studied. These compounds may be obtained upon the oxidation of 1-(pyrid-2-yl)-3-aryltetrazenes by 2,4,4,6-tetrabromocyclohexadien-1-one [2].



1,3,5- and 1,4,5-Trisubstituted Tetrazolium Salts. The feasibility of obtaining tetrazolium salts upon the alkylation of disubstituted tetrazoles was first reported by Benson et al. [3]. This reaction has subsequently been commonly used as a general method for the preparation of 1,3,5- and 1,4,5-trisubstituted tetrazolium salts.

Selectivity is the key problem in the alkylation of disubstituted tetrazoles. The ratio of the products of the alkylation of tetrazoles is independent of the properties of the reaction medium but is a function of nature of the substrate, alkylating re-

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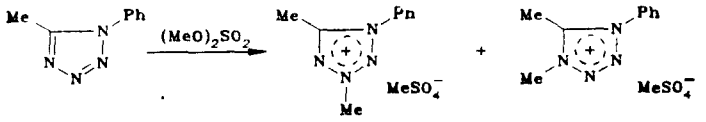
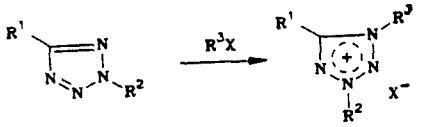
agent, and temperature. For example, alkylation in the reaction of 1-methyl-5-aryltetrazoles with dimethyl sulfate at 25°C proceeds at $N_{(4)}$ in the heterocycle, at which the most complete electron charge is concentrated [7]. The alkylation of 1-methyl-5-aryltetrazoles under the same conditions but at 60°C leads to 2,4-dimethyl-5-aryltetrazolium salts. At higher temperatures, the initially formed 1,4-dimethyl-5-aryltetrazolium salts isomerize to the more stable 2,4-dimethyl derivatives. A 16:84 mixture of 1-phenyl-3,5-dimethyl- and 1-phenyl-4,5-dimethyltetrazolium salts is formed upon the alkylation of 1-phenyl-5-methyltetrazole by dimethyl sulfate at room temperature [8].

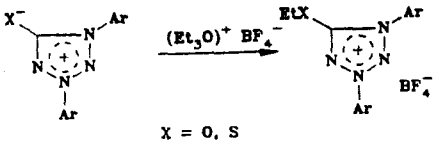


Upon replacing dimethyl sulfate with triethyloxonium tetrafluoroborate, the percentage of the corresponding 1,3,5-trisubstituted tetrazolium salt in the alkylation products increases to 25% [8]. Examples have been reported in which equal amounts of isomeric 1,3,5- and 1,4,5-trisubstituted tetrazolium salts are formed upon the alkylation of disubstituted tetrazoles. Thus, the alkylation of 1-methyl-5-methylthiotetrazole by tert-butyl alcohol in the presence of tetrafluoroboric acid leads to a 49:51 mixture of the corresponding 1,3,5- and 1,4,5-trisubstituted tetrazolium salts [9]. The alkylation of 1-alkyltetrazoles by trimethyloxonium tetrafluoroborate, of 1,5-disubstituted tetrazoles by dimethyl sulfate or bromoacetophenone, and of fused 1,5-disubstituted tetrazoles by dimethyl sulfate or methyl iodide occurs mainly at $N_{(4)}$ of the heterocycle [10-12].

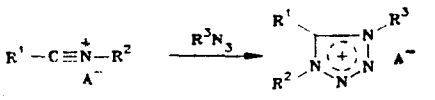
2,5-Disubstituted tetrazoles are less reactive in alkylation reactions than the isomeric 1,5-disubstituted derivatives [7]. On the other hand, independently of the nature of the substituents and alkylating reagent, 2,5-disubstituted tetrazoles are alkylated to give 1,3,5-trisubstituted tetrazolium salts [7, 8, 11]. Analogously, the corresponding 1,3,5-trisubstituted tetrazolium salt is formed upon the alkylation of 2-acetonyl-5-methyltetrazoles by methyl fluorosulfonate and methyl iodide at high pressure [13].



Finally, we should note that mesoionic tetrazoles may be used as starting reagents for the preparation of 1,3,5-trisubstituted tetrazolium salts. For example, the corresponding tetrazolium salts are formed upon the alkylation of 1,3-diaryl-5-tetrazolones or 1,3-diaryl-5-tetrazolethiones in good yields [14]:



Tetrazolium salts may also be obtained upon the reaction of nitrilium salts with alkyl and aryl azides. It is quite significant that 1,4,5-trisubstituted tetrazolium salts are the only products in this case [15].

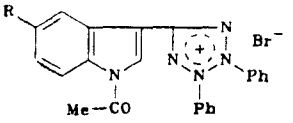


2,3,5-Trisubstituted tetrazolium salts. As already noted, the only method for the preparation of 2,3,5-trisubstituted tetrazolium salts is the oxidation of 1,3,5-trisubstituted formazanes.

A very broad range of oxidizing agents, including nitrogen oxides, nitrous acid, mercury salts, chromium(III) oxide, lead dioxide, halogens, alkyl nitrites, lead tetraacetate, haloimides, hydrogen peroxide, and atmospheric oxygen, has been used for this reaction [5]. The range of compounds used for oxidizing formazanes is rather broad. Nevertheless, none of these oxidizing agents may be seen as a universal reagent for obtaining tetrazolium salts.

The use of such standard oxidizing agent such as nitrous acid, alkyl nitrites, and lead tetraacetate generally gives good results. Thus, tetrazolium salts are formed in 55-92% upon the oxidation of 1,3,5-triarylformazanes by nitrous acid in acetic acid with subsequent treatment of the reaction mixture with perchloric acid [16]. Under these conditions, the corresponding tetrazolium nitrates were identified as intermediates. The experimental results indicated that atmospheric oxygen participates in the oxidation reaction. In the case of 1-(methyltetrazol-5-yl)- and 1-(2-methyltetrazol-5-yl)-3-phenyl-5-arylformazanes, Shchipanov [17] showed that nitrous acid may be used for the oxidation of hetarylformazanes to the corresponding tetrazolium salts. A number of authors have noted the high efficiency of reagents such as alkyl nitrites. Tetrazolium salts are formed in 50-98% yield upon the oxidation of 1,5-diaryl-3-acetyl- and 1,3,-diaryl-5-selenazol-2-ylformazanes by isoamyl nitrite [18, 19]. Some workers still prefer such oxidizing agents as lead tetraacetate. The use of this reagent is advantageous in the step involving separation of the tetrazolium salt [18, 20].

We should stress that a considerable number of studies have recently appeared in which the oxidation of various formazanes using N-haloimides and halogens has been described. The oxidation of formazanes by N-haloimides usually proceeds under mild conditions. This circumstance is highly significant when the substrate lacks thermal and chemical stability. The use of N-haloimides is most advantageous in the oxidation of hetarylformazanes. Thus, the reaction of indolylformazanes with N-bromosuccinimide gives tetrazolium salts (Ia) and (Ib) [21].

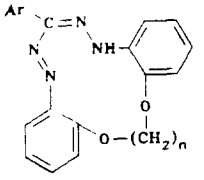


I a,b

I a R=H; b R=F

A significant effect of the nature of the substituent in the indolyl fragment of the formazane substrate on the yield of the tetrazolium salt was noted in a study of this reaction. For example, the yield of the tetrazolium salt was increased from 41 to 83% upon replacing hydrogen with fluorine. Analogously, the oxidation of the corresponding formazanes using N-bromosuccinimide gave a series of tetrazolium salts, containing thiazolyl, benzothiazole, and alkylbenzimidazole substituents [22, 23].

Several examples of the oxidation of complex formazanes using N-bromosuccinimide have been described. In particular, the corresponding tetrazolium salts were obtained upon the oxidation of formazanes (II) in ethanol or chloroform [24]. Ethyl acetate may be used as the solvent in the oxidation of formazanes by N-haloimides [25].

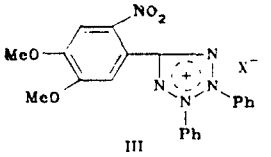


II n=1-6

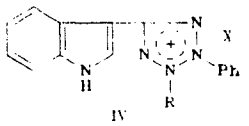
2,3,5-Triaryltetrazolium salts are obtained in 86-92% yield upon the oxidation of 1,3,5-triarylformazanes using gaseous chlorine in absolute ethanol [26, 27]. Ditetrazolium salts may be obtained by analogous procedures [28]. However, the use of gaseous chlorine and absolute ethanol significantly reduces the value of this method. Bromine is a less efficient oxidizing agent for triarylformazanes and permits the preparation of tetrazolium salts in 76-78% yield [29]. In a study of the kinetics of the oxidation of triarylformazanes by bromine, Hegarty et al. [30] found that the reaction has overall second-order kinetics and is first-order relative to each of the reagents. The oxidation rate depends on the nature of the substituents in the phenyl rings of the formazane. The presence of electron-donor substituents leads to an increase in the reaction rate, while electron-withdrawing substituents lead to a rate decrease. The strong effect of the dielectric constant of the solvent on the oxidation rate indicates an ionic mechanism. The same conclusions were later obtained in a study of the oxidation of triarylformazanes using thallium(III) acetate [31, 32]. The mechanism proposed by Hegarty [30] involves electrophilic attack of bromine at the

carbon atom of the formazane fragment, loss of a proton from $N_{(1)}$, and cyclization with elimination of a bromide ion. Despite some similarity of the proposed mechanism for the oxidation of formazanes in its first step with the mechanism for the bromination of hydrazones, it remains unclear why the electrophilic attack proceeds at the carbon atom and not at $N_{(1)}$, which has higher electron density.

In a discussion of the methods for the oxidation of formazanes to give tetrazolium salts, we should indicate the methods holding practical importance. Such methods include the oxidation of formazanes by hydrogen peroxide and oxidation using potassium permanganate and other oxidizing agents in two-phase systems. A special feature of the oxidation of formazanes by hydrogen peroxide is the requirement of a catalyst, namely ferrous ions or vanadium(V) oxide. As an example, let us take the case of the use of hydrogen peroxide containing a catalytic amount of ferrous salts in the preparation of salts with aryl (III) and hetaryl substituents (IV) [33-35]. Misra and Dhar [36] noted that hydrogen peroxide in some cases is a more efficient oxidation agent for formazanes than the reagents usually employed for this purpose.



III



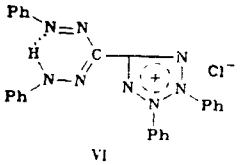
IV

In recent yers, a number of studies have been carried out on the oxidation of 3-alkyl-1,5-diaryl- and 1,3,5-triarylformazanes in two-phase systems in regard to the development of phase transfer catalysis [37-40]. These formazanes in a two-phase system consisting of methylene chloride or chloroform and water are smoothly oxidized by potassium permanganate to the corresponding tetrazolium salts. This reaction proceeds rapidly at 10-15°C and is complete 15-20 min after mixing of the reagents. The tetrazolium salt yields are 70-87%. In a study of this reaction, the oxidation of formazane was found to proceed by a phase transfer catalysis mechanism. The tetrazolium salt formed in the first oxidation step acts as the phase transfer catalyst in this reaction. Similar results were obtained in the oxidation of triarylformazanes in a two-phase system by nitrous acid [41] and under the same conditions with treatment of the formazanes by thionyl chloride [42].

We should note that these methods for the oxidation of formazanes have not been sufficiently developed. Nevertheless, the use of hydrogen peroxide, which is one of the most ecologically "clean" oxidizing agents, and the oxidation of formazanes under phase transfer catalysis conditions may be considered to hold the greatest practical promise for the preparation of tetrazolium salts. In addition to the indicated methods for the synthesis of tetrazolium salts, we should note the electrochemical oxidation of formazanes in acetonitrile [43]. In this case, the tetrazolium salt yield is 95-100%. In conclusion, we note that the methods for the preparation of mesoionic tetrazoles have been described in a review by Newton and Ramsden [44].

PHYSICOCHEMICAL PROPERTIES

Crystal and Electronic Structure. Crystal structures have been determined for two tetrazolium salts with "ordinary" structure, namely 2,3-diphenyl-5-methylthiotetrazolium triiodide (V) and 1,3-diphenyl-5-(1,5-diphenyl-3-formazyl)tetrazolium chloride (VI) [45, 46]. The bond lengths in V between the atoms in the tetrazolium cations range from 0.130 to 0.141 nm. The angles between the plane of the tetrazole ring and the phenyl rings are 69° (2-phenyl group) and 58° (3-phenyl group). The iodine atoms in the anion form an angle of 177° with bond lengths of 0.294 and 0.290 nm, while the anions themselves are arranged in chains within the crystal. The 1-phenyl and 3-phenyl rings in VI are twisted from the tetrazole ring plane. The bond lengths in the tetrazole ring fall in the narrow range from 0.130 to 0.136 nm. The chloride ion is located above the plane of the tetrazole ring. The distances from this anion to the nitrogen and carbon atoms of the ring are 0.341 (N_1), 0.362 (N_2), 0.370 (N_3), 0.365 (N_4), and 0.344 (C_5).

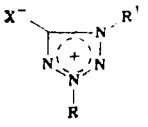


VI

The crystal structures of various mesoionic tetrazoles have been studied in considerable detail. Structural analyses have been carried out for 1,3- and 2,3-diphenyltetrazolium-5-thiolates (VII) and (VIII) and for 2,3-diphenyltetrazolium-5-olate (IX) [47, 48]. The bond lengths between the tetrazole ring range from 0.129 to 0.139 nm (VII) and from 0.131 to 0.138 nm (IX). The phenyl rings in both cases are extruded from the tetrazole ring plane. The crystal structure of VIII is analogous to the structure of IX.

A CNDO calculation yielded the electronic structure of the simplest tetrazolium cation, namely tetrazole protonates at $N_{(4)}$. There is a significant decrease in electron density on all the ring atoms in going from tetrazole to the tetrazolium cation [49]. The protonation of tetrazole has hardly any effect on the bond multiplicity, especially for the $N_{(1)}$-$N_{(2)}$ bond. Similar behavior is found for the electronic structure of 1- and 2-methyltetrazoles and 1,3-, 1,4-, and 2,3-dimethyltetrazolium cations [50]. The greatest positive charge in the 2,3-dimethyltetrazolium cation is found at $N_{(2)}$ and $N_{(3)}$ in the heterocycle. These results are in good accord with the experimentally observed cleavage of the $N_{(2)}$-$N_{(3)}$ bond in 2,3,5-triaryltetrazolium salts upon their chemical and electrochemical reduction.

Electronic Spectra. Mono- and ditetrazolium salts have strong ultraviolet absorption with a maximum at 240-280 nm (log ε 4.28-4.88) [51, 52]. The presence of a dimethylamino group in the phenyl ring at $N_{(2)}$ of the heterocycle leads to the appearance of a band at 440 nm in the spectrum of the 2,3,5-triaryltetrazolium salt. This band is shifted to 472-475 nm upon the additional introduction of a nitro group into the phenyl ring at $N_{(3)}$ [16]. The long-wavelength UV maximum in mesoionic tetrazoles X is shifted to 326-333 nm [14]. This maximum is shifted to 475-565 nm for 1,3-diphenyl-5-cyclopentadienyltetrazolium salts depending on the nature of the solvent [53]. The presence of an electron-withdrawing substituent at $N_{(3)}$ in the heterocycle in the case of 2-(tetrazol-5-yl)-3,5-diaryltetrazolium salts leads to a bathochromic shift of the absorption band and the additional introduction of an electron-withdrawing substituent into the phenyl ring at $C_{(5)}$ even further intensifies this effect [54, 55].



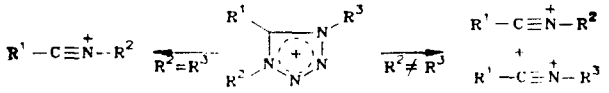
X X=S,O

NMR Spectra. NMR spectroscopy is rather commonly used for the identification of isomeric tetrazolium salts. ^1H NMR spectral studies of 1,4,5- and 1,3,5-trisubstituted tetrazolium salts have indicated that the signals of the methyl group protons at $N_{(1)}$, $N_{(4)}$, and $C_{(5)}$ in the heterocycle differ significantly, while the signals for the protons of the phenyl groups at the same positions of the tetrazole ring fall in the same region. The signals of the protons of the methyl groups at $N_{(1)}$, $N_{(4)}$, and $N_{(2)}$ in the heterocycle also differ slightly, which permits the reliable identification of the corresponding isomeric tetrazolium salts [7, 8]. Analogously, the symmetrical 1,4-diethyl- and 2,3-diethyl-5-phenyltetrazolium salts and asymmetrical 2,4-diethyl-5-phenyltetrazolium salt differ slightly [15, 56]. Konnecke and Lippman [57] used ^1H and ^{13}C NMR spectroscopy in an attempt to identify isomeric 1-ethyl-3-phenyl- and 1-ethyl-4-phenyl-tetrazolium salts. For this purpose, the NMR spectra of these compounds in various solvents were studied. However, change in the nature of the solvent had only a slight effect on the difference in the chemical shifts of the ortho protons of the phenyl rings at $N_{(3)}$ and $N_{(4)}$ of the heterocycle.

Mass Spectra. The fragmentation of isomeric tetrazolium salts and mesoionic tetrazoles upon electron impact proceeds by characteristic pathways and leads to the formation of various fragment ions [58-60]. Analysis of the composition and intensities of the fragment ion peaks permits unequivocal determination of the structure of tetrazolium salts.

The mass spectra of 2,3-diphenyl-5-methyltetrazolium tetrafluoroborate and 2,3,5-triphenyltetrazolium bromide have been studied in considerable detail [58]. The mass spectrum of 2,3-diphenyl-5-methyltetrazolium tetrafluoroborate obtained at 70 eV shows the molecular ion peak at m/z 256 and tetrazolium cation peak at m/z 237. The predominant fragmentation pathway of the molecular ion involves formation of the $C_6H_5N_2^+$ ion at m/z 105 and the complementary ion at m/z 132 formed upon cleavage of the tetrazole ring. The strongest peak in the mass spectrum corresponds to the $C_6H_5N^+$ ion at m/z 91, which is formed as a result of the elimination of acetonitrile from the ion with m/z 132. The low-intensity peaks at m/z 151 and 110 correspond to fluorine-containing fragment ions $C_8H_8N_2F$ and C_6H_5NF, respectively. Messmer et al. [58] detected ions in the mass spectrum formed from the tetrazolium cation and fluorine atom and postulated the formation of an N–F bond. We should note that the mass spectrum is markedly simplified with a reduction in the ionizing radiation energy to 10 eV. In this case, the peak at m/z 237, corresponding to the tetrazolium cation, is the most intense, while the intensity of the peak at m/z 91 is significantly reduced.

Carbony et al. [59] presented the mass spectra of 1,4,5-trisubstituted tetrazolium salts and indicated the pathways for the fragmentation of the tetrazolium cations formed as a result of the elimination of a radical from 1,4,5-trisubstituted tetrazolines. The tetrazolium ion undergoes fragmentation through one or two pathways depending on whether the substituents at the nitrogen atoms are the same or differ:



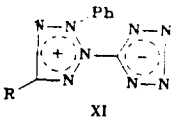
The mass spectra of mesoionic tetrazoles have been studied in detail by Hanley et al. [60]. Simultaneous decomposition along all four pathways never occurs.

Thermal Stability. The data on the thermal stability of tetrazolium salts are extremely limited. Derivatography has recently been employed to study the stability of 2,3,5-triphenyltetrazolium chloride, bromide, and iodide [40]. The DTA curves of these compounds show two exothermal peaks at 215-232 and 450-500°C. Rapid decomposition of all the tetrazolium salts studied independently of the nature of the halide anion begins at 215-225°C. Hence, tetrazolium salts have rather high thermal stability, which is apparently a function of the thermal stability of the heterocycle.

CHEMICAL PROPERTIES

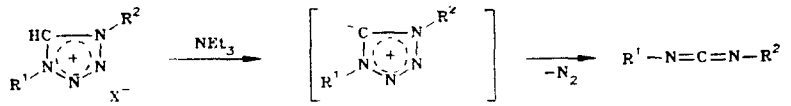
Although tetrazolium salts have been known for almost 100 years and commonly used in scientific research, medicine, and agriculture, the chemical properties of these compounds have not been studied systematically. The chemical transformations of tetrazolium salts holding the greatest theoretical and practical interest are examined below.

Action of Acids. The stability of tetrazolium salts to the action of mineral acids depends mainly on the nature of the substituents on the tetrazole ring. Thus, selective sulfonation of the phenyl substituents occurs upon the treatment of 2,3,5-triphenyltetrazolium chloride by concentrated sulfuric acid or oleum with the formation of the corresponding disulfonic acid derivative [5]. On the other hand, the formation of the corresponding 2,5-disubstituted tetrazoles occurs upon the action of mineral acids on tetrazolium salts containing heterocyclic substituents at $N_{(3)}$ of the tetrazole ring. Sedov [20] and Lipunova [23] demonstrated this behavior in the case of tetrazolium salts containing benzimidazole, benzothiazole, and benzoxazole substituents. It is interesting that tetrazolium salts cannot be isolated upon the oxidation of 1-(pyrimidin-2-yl)-3-phenyl-5-arylformazanes by N-bromosuccinimide in acetic acid since such tetrazolium salts are converted under the reaction conditions to the corresponding 2-aryl-5-phenyltetrazoles [61]. Tetrazolium salts containing tetrazolyl substituents are more resistant to the action of mineral acids and their decomposition requires heating at reflux in hydrochloric acid (100°C) [62]. Under these conditions, mesoionic tetrazoles (XI) are not altered at all [63]:



XI

Action of Bases. It was noted even in the first studies on 2,3,5-trisubstituted tetrazolium salts that these compounds are unstable in basic media [5, 6]. However, the mechanism of this reaction has hardly been studied. In a study of the kinetics of the decomposition of 2,3,5-triphenyltetrazolium hydroxide, the reaction was shown to be first-order relative to the 2,3,5-triphenyltetrazolium cation and second-order relative to the hydroxide ion. One of the reaction products is 1,3,5-triphenylformazane [64]. In concentrated alkali solutions, 2,3,5-triphenyltetrazolium chloride undergoes more complex transformations analogous to those occurring upon the UV irradiation of alcoholic solutions of the salt, which gives 2-phenyltetrazolo[2,3-α]dibenzo[c,e]pyridazinium chloride (2,3-diphenylene-5-phenyltetrazolium chloride) [65].

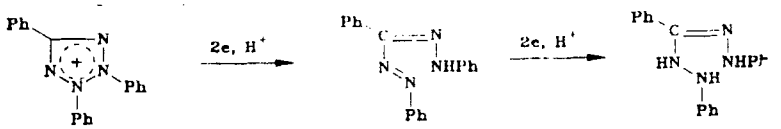


1,4-Di- and 1,4,5-trisubstituted tetrazolium salts undergo other transformations upon the action of base. Thus, the reaction of 1,4-disubstituted tetrazolium salts with triethylamine and other tertiary aliphatic amines leads to deprotonation of

the tetrazole ring with its subsequent opening, the loss of nitrogen, and carbodiimide formation [10, 66]. 1,4,5-Trisubstituted tetrazolium salts undergo dealkylation upon the action of tertiary amines and sodium hydroxide with the formation of 1,5-disubstituted tetrazoles [66].

Action of Reducing Agents. 2,3,5-Trisubstituted tetrazolium salts are readily reduced to the corresponding formazanes [5]. Hydroxylamine, hydrazine, ammonium sulfide, and ascorbic acid may be used as the reducing agents. The use of more active reducing agents leads to the destruction of resultant formazane. This reaction proceeds in neutral or slightly basic media.

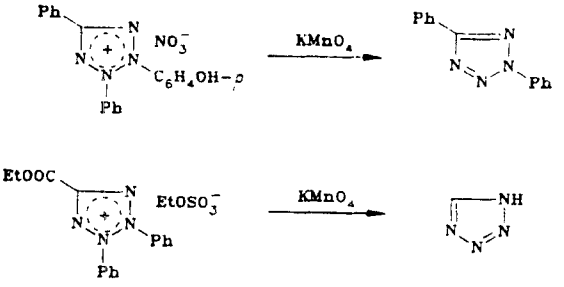
The electrochemical reduction of 2,3,5-trisubstituted tetrazolium salts has been studied extensively. The electrochemical reduction of tetrazolium salts in neutral and basic aqueous or aqueous organic solvents proceeds by the following scheme [67, 68]:



On the other hand, the reduction in acid media proceeds directly to benzhydrazine, bypassing the step involving formazane formation.

A detailed study of the oxidation-reduction transformation of 1,3,5-triphenylformazane to the 2,3,5-triphenyltetrazolium cation indicates that this reaction proceeds with the transfer of two electrons and one proton. A tetrazolyl radical is the intermediate upon the tetrazolium salts [43, 69]. The results of a polarographic study of substituted 2,3,5-triaryltetrazolium salts indicates that the redox potential of these compounds is virtually independent of the nature of the substituents in the phenyl rings [67]. This finding may be attributed to the circumstance that the phenyl substituents at $N_{(2)}$ and $N_{(3)}$ of the tetrazole ring lie outside the plane of the heterocycle. On the other hand, the introduction of a nitro group to the phenyl group at $C_{(5)}$ leads to a reduction in the reduction potential of a tetrazolium salt by about 300 mV. However, the effect of other substituents on the redox potential of these salts is only slight [43].

Other Reactions. Tetrazolium salts are rather stable to the action of oxidizing agents. On the other hand, cases have been reported, in which 2,5-disubstituted tetrazoles or unsubstituted tetrazole are formed upon the oxidation of tetrazolium salts by potassium permanganate in nitric acid soluiton [70]:

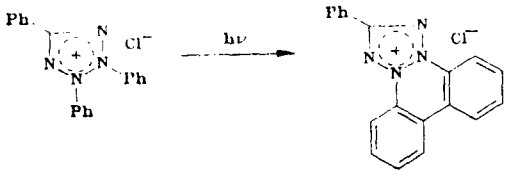


The methyl group hydrogen atoms in 1,3,5-trimethyl- and 1,4,5-trimethyltetrazolium salts are rather readily replaced by deuterium [71].

A number of workers have noted the high reactivity of mesoionic tetrazoles, which are converted into "ordinary" tetrazolium salts upon the action of triethyloxonium tetrafluoroborate or methyl iodide [72].

2,3,5-Trisubstituted tetrazolium salts have high light sensitivity. Photochemical reduction, sensitized photoreduction, and photochemical oxidation have been reported for these compounds. The formation of 2,3,5-triphenylformazane is observed upon the irradiation of alkaline aqueous solutions of 2,3,5-triphenyltetrazolium chloride with visible light [73, 74]. Such processes proceed more efficiently upon the introduction of sensitizers, which absorbs visible light and reduces the tetrazolium salt in the presence or absence of an electron donor [75]. The reduction of tetrazolium salts by the superoxide ion [76] and 2-isobutylanthraquinone radical, generated photochemically under the experimental conditions, has also been studied [77].

The photochemical oxidation of 2,3,5-trisubstituted tetrazolium salts proceeds upon UV irradiation [78, 79]. The direction of this reaction depends predominantly on the nature of the solvent. 2,3,5-Triphenyltetrazolium chloride gives 1,3,5-triphenylformazane when the reaction is carried out in aqueous solution. When water is replaced by ethanol, 2,3,5-triphenyltetrazolium chloride is converted to 2-phenyltetrazolo[2,3-*a*]dibenzo[c,e]pyridazinium chloride (2,3-diphenylene-5-phenyltetrazolium chloride) in quantitative yield [79].



APPLICATIONS

There is no information on the practical application of isomeric 1,2,5-, 1,3,5-, and 1,4,5-trisubstituted tetrazolium salts. However, there is evidence to consider that these compounds may be used as analytical reagents and phase-transfer catalysts. 2,3,5-Trisubstituted tetrazolium salts have found common use in biochemistry, microbiology, histochemistry, and cytochemistry as extraction agents in the separation and determination of metals, as photosensitive compounds or image-forming compounds, and in various data recording systems. The potentials of the redox transformations of 2,3,5-trisubstituted tetrazolium salts are similar to the potentials of oxidation–reduction enzymes of living organisms and the redox potentials of electron acceptors in the photosynthetic apparatus of plants. This permits the common use of tetrazolium salts in biochemical as indicators of the oxidation–reduction activity of enzymes [6, 80, 81] and as electron acceptors in studying photosynthesis processes [82, 83]. The use of tetrazolium salts as indicators in microbiology is based on the capacity of these compounds to trap electrons, which respiring organisms may transfer to elemental oxygen or some other natural electron acceptors. The tetrazolium salts are reduced in this case to formazane [6]. The use of tetrazolium salts as indicators in histochemistry and cytochemistry is based on the same principle, namely the reduction of tetrazolium salts to formazanes [84, 85]. Tetrazolium salts are so commonly used for the determination of hydrogenases that the corresponding methods are given in virtually all histochemistry textbooks. The number of studies devoted to the determination of various biochemical objects using tetrazolium salts is very great and vastly exceeds the number of studies on the synthesis and properties of these compounds. 3,3'-(3,3'-Dimethoxy-4,4'-biphenylene)bis[5-phenyl-2-(4-nitrophenyl)tetrazolium chloride], whose applications have summarized in reviews by Feigin [86] and Morse [87], is especially common.

Tetrazolium salts are used as indicators for the quantitative determination of ferrous ions [88], hydrogen sulfide [89], hydrazides of aromatic carboxylic acids [90], dipyrone [91], glucose [92], and steroids [93].

The reduction of tetrazolium compounds is the basis of various applications of these compounds in information recording systems. Of the entire range of such systems, we should note systems in which the image arises as a result of the reaction of the tetrazolium salt with a photogenerated reducing agent [94, 95], and those in which the tetrazolium salts are used as a component of developing compositions [96]. Photosensitive compositions based on polymers and copolymers containing tetrazolium salt fragments have been patented [97, 98]. We should also note the use of tetrazolium salts as components for the preparation of ionizing radiation dose meters [99, 100] and indicators for the germinating capacity of grains [24]. Finally, it has most recently been shown that tetrazolium salts may be used as phase transfer catalysts in oxidation and acylation reactions and the formation of acyl azides [37, 40, 101, 102].

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