THE CHEMISTRY OF FORMAZANS AND TETRAZOLIUM SALTS

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I. Introduction

This review aims at providing a general survey of the chemistry of formazans and of tetrazolium salts. As all tetrazolium salts are prepared, directly or indirectly, from formazans, it seems logical to consider the two classes of compounds together, although the immediate structural relationship may not be apparent on paper. A brief discussion of the biological activity shown by these compounds is included under Section XIV. Tables of the known compounds, with their physical properties, are appended. The review covers the literature to the end of 1952 and describes much of the work reported during 1953.

A short review of this subject, published in German by Ried (152), deals with recent biological applications of tetrazolium salts. A brief earlier survey by Smith (172) is also essentially concerned with the use of tetrazolium compounds in biochemical and medical research. Jerchel (99) has described German work in this field during the 1939–1945 period.

Although these compounds were first described in the last decade of the

nineteenth century, they have been studied extensively only since 1941, the year in which Kuhn and Jerchel drew attention to their possible value in biochemical research. Since that date, an increasing flow of work has appeared on the chemistry of new types of these compounds and on their biochemical application. It seems opportune, therefore, to survey the broader aspects of their chemistry, since this has not previously been done in any detail.

II. Definition of Formazans and Tetrazolium Salts

A. FORMAZANS

Formazans are those compounds which contain the characteristic chain of atoms $-N{=}N-\overset{|}{C}{=}N-NH-$. Such compounds form a distinct class with characteristic properties; their structure was first elucidated by Bamberger (25) and by von Pechmann (32), who agreed to call them "formazyl compounds."

The term *guanazyl* is applied to variations of this structure in which the $=N-NH-$ group is linked to a guanyl group and not to an aryl radical (as it is in all ordinary formazans hitherto described). Such compounds are characterized by the group

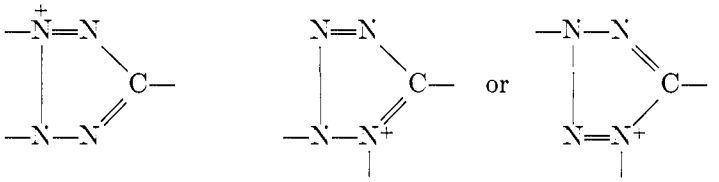
$$-N{=}N-C{=}N-NH-C(=NH)-NH_2$$

They are sufficiently akin to the formazans to be described in this review, but their chemistry is not extensive.

Recently two further types of compounds of related structure have been described: (*a*) In one a cholyl radical (introduced as a cholylhydrazide) replaces the aryl group normally attached to the $-NH-N=$ system. (*b*) In the other a diaryl carbonyl group (introduced as a diarylsemicarbazone) replaces the same aryl group.

B. TETRAZOLIUM SALTS

As their name implies, tetrazolium salts are quaternized tetrazoles and therefore contain a ring of one carbon and four nitrogen atoms, one of which is quaternary. As a result, the compounds have salt-like properties.



I II

Almost all the known tetrazolium salts (I) are derived from (2*H*)tetrazole, although the series (II) derived from (1*H*)tetrazole is theoretically possible. (Hünig and Boes (93) have recently described compounds of this type. They have suggested that the endoxytetrazole compounds described by Busch and Schmidt (47) belong to this class. These compounds are discussed in more detail on page 408.) There remains one tetrazolium salt of unascertained structure,

obtained by the action of methyl iodide on 5-methyl-1-(3,4-dimethylphenyl)-tetrazole (32).

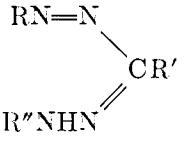
Although a wide variety of *C*-substituents has been introduced, every tetrazolium salt so far reported has aryl groups attached to the nitrogen atoms; this is inherent in the synthetic methods available.

The close structural relationship of tetrazolium salts to tetrazoles is unimportant in practice because the preparative methods for the two classes are very different. The biological activity of the two classes also appears to be quite distinct. A review of the tetrazoles by Benson (31) does not discuss tetrazolium salts.

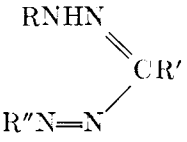
III. Nomenclature

A. Formazans

Difficulties in the nomenclature of these compounds have resulted from divergences in British, American, and German practice. The two general structures (IIIa and IIIb) for a formazan are chemically indistinguishable (see page 364 *et seq.*) and lead to two possible names for each individual formazan when R and R″ are not alike.

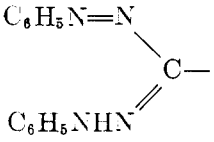


IIIa

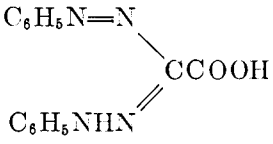


IIIb

The first workers to appreciate the nature of these compounds based their structure on the radical



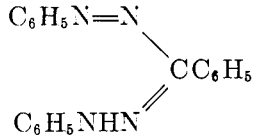
which they called formazyl. IV was therefore called formazylcarboxylic acid.



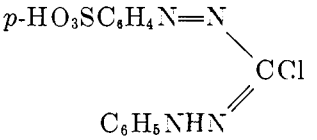
IV

This terminology is retained by Mitchell in his monograph *British Chemical Nomenclature* (123), and "formazyl" is listed among other radicals in the nomenclature report of *Chemical Abstracts* (128). The method is open to the serious objection that it is impossible to name compounds in which the *N*-phenyl groups are replaced by others which cannot be named as substituted phenyls (naphthyls, pyridyls, etc.). Molecules containing two formazan groups also lie beyond the

scope of this nomenclature. No consistent method has appeared in recent papers published in America or in *British Chemical Abstracts*. The indexers of *Chemical Abstracts* have applied a terminology which treats the formazans as phenylhydrazono derivatives of azo compounds, so that the original term "formazylbenzene" for V becomes phenylazo-β-phenylhydrazonotoluene.



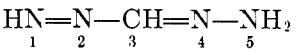
V



VI

This represents the structure of these compounds inaccurately (see the discussion on formazan tautomerism on page 364) and becomes very obscure in the case of VI, which is referred to as *p*-[2-(chlorophenylazomethylene)hydrazino]benzenesulfonic acid.

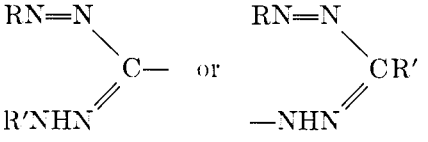
Modern German usage is exemplified by *Beilstein* (4th edition, Volume 16, 1933, introduction), in which the hypothetical fundamental compound



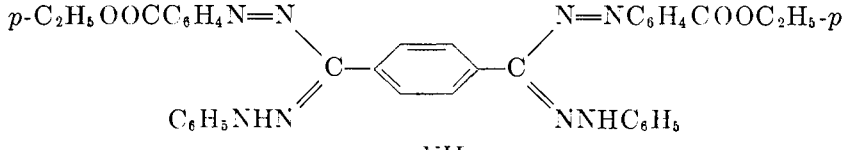
is termed formazan, so that V becomes 1,3,5-triphenylformazan and VI becomes 3-chloro-1-phenyl-5-*p*-sulfophenylformazan. Although the numbering may be applied arbitrarily in either direction, this cannot lead to confusion. This method has been adopted in the present work because of its greater versatility and accuracy in describing structures; it is concurrently in use in the *Journal of the Chemical Society* (2).

It may be noted that the oxa-aza system of nomenclature is applicable to formazans; the application of this system to aliphatic compounds has not been approved by the I.U.P.A.C. Committee on Nomenclature. It would merely involve the replacement of the long-established term "formazan" by "3-carbapentaaza-1,3-diene."

The term "formazyl" can be applied to disubstituted radicals of the types



so that diformazans can be named without difficulty. VII therefore becomes 1,4-bis(1-phenyl-5-*p*-ethoxycarbonylphenyl-3-formazyl)benzene.

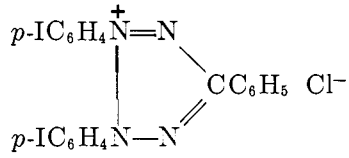


VII

In this connection, the suggestion of Rapoport and Bonner (151) with regard to the reduced compounds related to formazans seems worthy of support. If the basic structure $NH_2NHCH{=}NNH_2$ is termed hydrazidine, these compounds can be named by a procedure parallel to that for formazans. The still more reduced compounds of the type $NH_2N{=}CHNH_2$ are named amidrazones and can be readily fitted into the general pattern. It is proposed to use these terms in this review where necessary.

B. TETRAZOLIUM SALTS

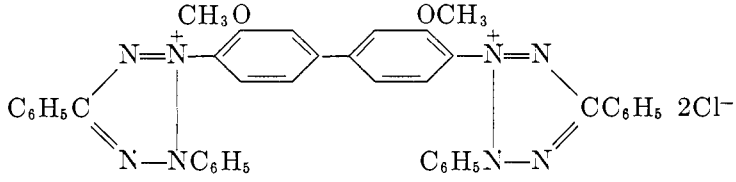
The naming of tetrazolium salts has caused less difficulty, and the American system has been followed in this review. VIII is therefore called 2,3-bis(4-iodophenyl)-5-phenyl(2*H*)tetrazolium chloride.



VIII

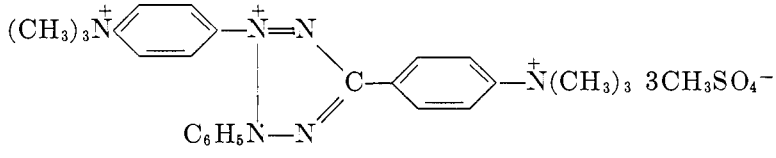
It is convenient and permissible to omit the "(2*H*)", which shows that the salt is formed by the quaternation of a 2-substituted tetrazole, since no (1*H*)tetrazolium salts are known with certainty.

Double tetrazolium salts are named in such a way that the name concludes with the term "tetrazolium salt." Thus IX is 3,3′-dimethoxy-4,4′-diphenylene-3″,3‴-bis(2,5-diphenyltetrazolium chloride).

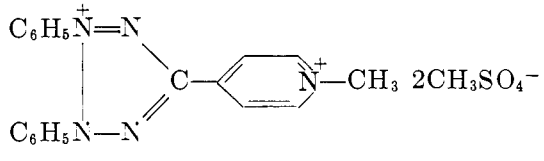


IX

Further difficulties arise when substituents which usually appear as suffixes are present in these compounds. A nomenclature which allows various types of quaternary groups to be named as prefixes is required.



X



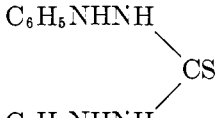
XI

Thus X and XI have had to be called 3,5-bis(*p*-dimethylaminophenyl)-2-phenyltetrazolium dimetho(methyl sulfate) methyl sulfate and 2,3-diphenyl-5,1′-methylpyridiniumtetrazolium di(methyl sulfate), respectively. The former name is manifestly obscurantist and the latter faces the difficulty of a compound containing two groups which can only be named as suffixes.[1]

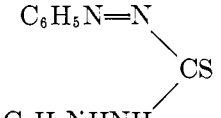
IV. Historical Survey

The first recorded preparation of a formazan was that of Friese (72), who reacted benzenediazonium nitrate with the sodium derivative of nitromethane. He regarded his product as phenylazonitromethane.

The condensation product of thiophosgene and phenylhydrazine (68, 69, 71) was shown to give diphenylthiocarbazide (XII), which was found to be oxidized readily to a red substance formulated as XIII.

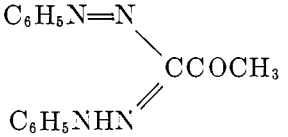


XII



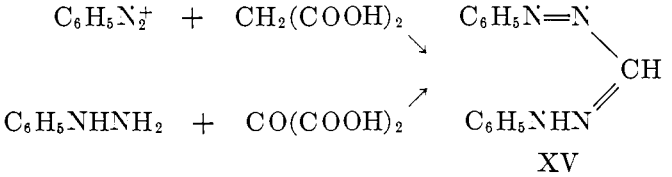
XIII

The corresponding oxygen analogs derived from phosgene were also described (85). Shortly after this the action of diazonium salts in alkaline solution on various aliphatic compounds containing active methylene groups was studied, and Claisen (57) suggested that the dark red solid isolated from the reaction between benzenediazonium chloride and benzeneazoacetylacetone might be XIV.



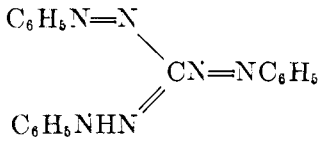
XIV

The problem was elucidated independently by von Pechmann (132) and Bamberger (25). The former showed that XV represented the structure of the product of the reaction of benzenediazonium chloride and malonic acid in alkaline solution, and that it was identical with the product formed by the action of phenylhydrazine on mesoxalic acid.



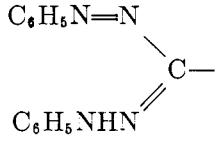
XV

[1] Recently, different workers have suggested the use of the "onio" prefix, as in trimethylammonio-, pyridino-, etc., as a means of circumventing this difficulty.



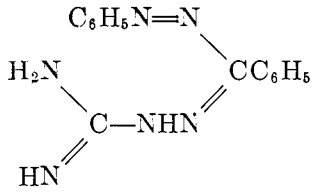
XVI

Bamberger (25) prepared XVI by treating acetoacetic acid, pyruvic acid phenylhydrazone, pyruvic aldehyde, acetone, and acetaldehyde separately with an excess of benzenediazonium chloride solution. These two workers then jointly proposed the name "formazyl", equivalent to



to describe compounds of this type. von Pechmann (132) showed that the condensation of diazonium salts with aldehyde phenylhydrazones in alkaline solution constituted a general method of formazan synthesis. He listed three main preparative methods (133). Further work followed on the tautomeric character of formazans (134, 135, 138) and on the products of their oxidation (138, 140). The latter were shown by von Pechmann and Wedekind (141, 180) to be tetrazolium compounds by the degradation of 2,3-bis(*p*-hydroxyphenyl)-tetrazolium chloride to tetrazole (see page 378).

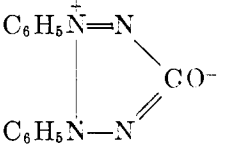
Thiele had earlier suggested that there was a close parallel between the properties of phenylhydrazine and those of aminoguanidine. This was confirmed by Wedekind (181, 189), who prepared guanazyls (e.g., XVII) by the condensation of diazonium salts with benzalaminoguanidines. He also showed that these compounds gave, on oxidation, tetrazoles and not tetrazolium salts.



XVII

Wedekind and Stauwe (191) studied the effect upon the ease of oxidation to tetrazolium salts when the phenyl groups attached to both carbon and nitrogen in the formazan molecule were substituted in various ways. Fichter and Schiess (66) prepared further formazans with novel substituents, in particular a number of those bearing sulfonic acid groups which rendered them soluble in water. These compounds were examined for their possible value as dyestuffs.

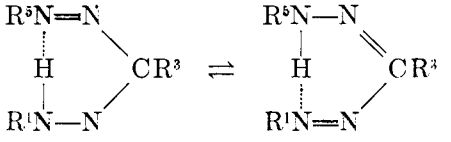
Although diphenylcarbazone and its sulfur analog (XIII) were first described many years before, they were studied more intensively from 1900 onwards and their relationship to formazans made clear. Oxidation gave the so-called "diphenylcarbadiazone," which Bamberger in 1911 (9) finally established as 5-hydroxy-2,3-diphenyltetrazolium betaine (XVIII).



XVIII

The chemistry of this and of 1,5-diphenylformazans bearing 3-amino, 3-nitro, and 3-mercapto groups was clarified to a great extent by the work of Bamberger, Padova, and Ormerod (20) in 1925. XVIII was not prepared in a pure state until 1937 (105).

The tautomerism of formazans, first described by Wedekind (134), was studied by Lapworth (113), who examined the mutarotation of optically active formazans; his results were inconclusive. In 1941 Hunter and Roberts (94) conclusively established for several pairs of formazans that the individuals in each pair, previously described as tautomeric, were identical. This they showed both by the properties of the free formazan and by those of certain of its metal derivatives. They suggested that formazans were resonance hybrids with a chelated hydrogen-bridge structure (XIX):



XIX

According to this formulation, therefore, there are no exactly located double bonds present. This view was advanced at the same time by Kuhn and Jerchel (106), who confirmed the mesomeric character of formazans by comparison of the absorption spectra and Debye–Scherrer x-ray diagrams of supposed pairs of formazans.

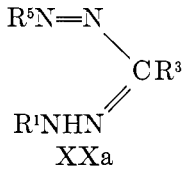
The corresponding tetrazolium salts were studied by the German workers, who laid the foundations of modern interest in this group of compounds by showing that they possessed valuable staining properties in various biological preparations and that they could, in particular, be used as indicators of the viability of seeds. They also found that some tetrazolium salts had a feeble bacteriostatic action (98, 106). During the years 1946–1948, Italian workers developed other aspects of formazan chemistry (74, 75, 147–150); since 1947 numerous papers, chiefly of American or German origin, have appeared which describe the biological applications of well-known tetrazolium salts. A foremost objective has been to develop new types of these compounds which show a differential staining of tissues and, in particular, to produce a tetrazolium salt which yields a blue stain (i.e., is reduced to a blue formazan). Ried and his coworkers (153, 154, 155, 156) have studied this problem intensively.

Kuhn (82, 109) has recently described investigations into the detailed structure of formazans. New types of substituted tetrazolium salts, in which the conjugation has been extended through a phenylazo or styryl group attached to *N*-phenyl rings, are reported by British workers (117, 127; tables 30 and 31).

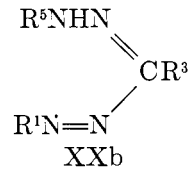
V. Molecular Structure of Formazans and Tetrazolium Salts

A. FORMAZANS

As has already been indicated, formazans of the two types XXa and XXb are indistinguishable.



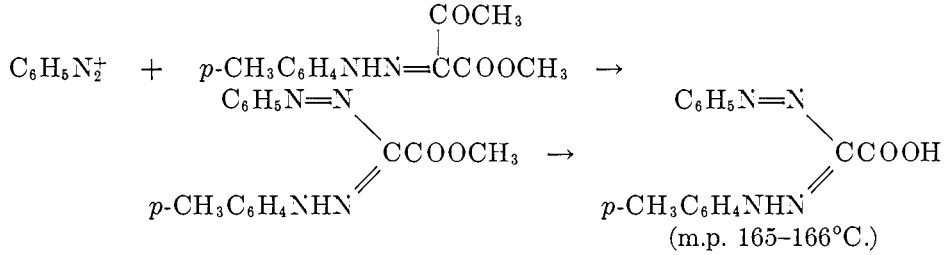
XXa



XXb

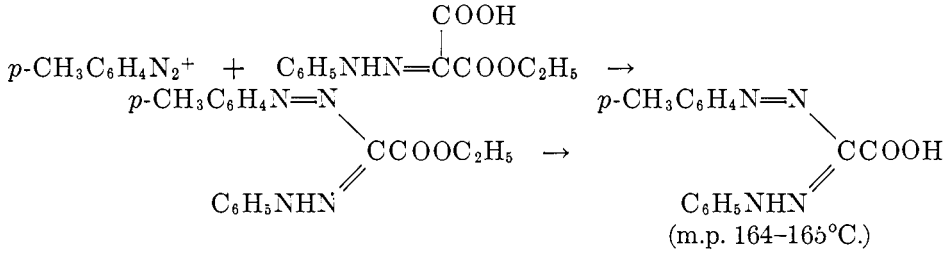
When the compound obtained by the condensation of the diazonium salt $R^5N_2^+X^-$ with the phenylhydrazone $R^1NHN{=}CHR^3$ is compared with the compound derived from the diazonium salt $R^1N_2^+X^-$ and the phenylhydrazone $R^5NHN{=}CHR^3$, they are found to be identical in appearance, melting point, solubility, and other properties. When these two compounds are made to undergo certain reactions, each yields the same single product; in other reactions, a mixture of the products which could theoretically be expected from each of the two formulations XXa and XXb is obtained. von Pechmann (134, 135, 138) therefore concluded that tautomerism was exhibited by formazans, and that it was of the type which he had previously studied in the aminoazo compounds and amidines, in which the mobile hydrogen atom is attached to two nitrogen atoms in turn. When an asymmetrical formazan (i.e., one in which the substitutents R^1 and R^5 are not alike) is acylated at the imide nitrogen atom, two different compounds result, which can be separated from each other fairly readily.

von Pechmann (134) prepared 3-methoxycarbonyl-1-phenyl-5-*p*-tolylformazan by the condensation of benzenediazonium chloride with methyl acetoacetate *p*-tolylhydrazone, and hydrolyzed it (with ethanolic sodium hydroxide solution) to the free acid.



(m.p. 165–166°C.)

He also condensed *p*-toluenediazonium chloride with ethyl hydrogen mesoxalate phenylhydrazone and showed that the 3-ethoxycarbonyl-5-phenyl-1-*p*-tolylformazan produced could be hydrolyzed to the same acid, m.p. 164–165°C.

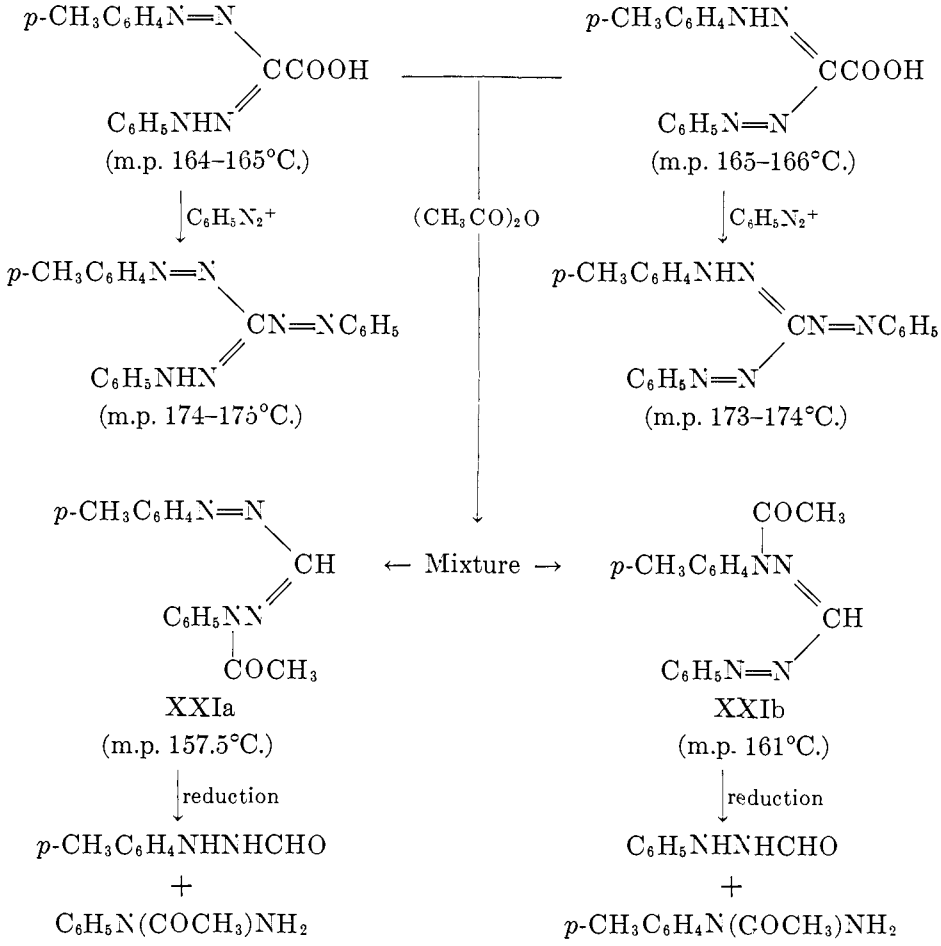


(m.p. 164–165°C.)

Both samples of this acid were reacted with acetic anhydride, and both gave, with loss of carbon dioxide, the same product, which was readily separated by crystallization into XXIa and XXIb. XXIa crystallized in the form of prisms, m.p. 157.5°C., and XXIb as needles, m.p. 161°C. These were identified by reductive hydrolysis (see the diagram below). Both XXIa and XXIb yield the one product when the acetyl group is hydrolyzed to give the parent formazan, and when this product is reacetylated, the same two forms can once more be isolated.

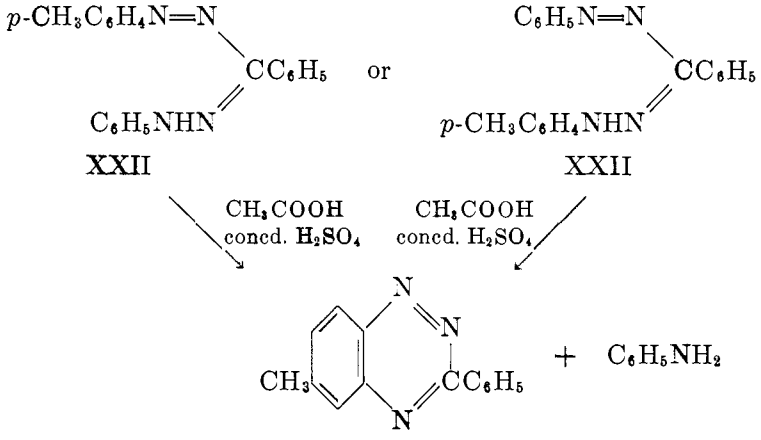
The two formazancarboxylic acids (m.p. 164–165°C. and 165–166°C.) were also shown to give identical samples of 1,5-diphenyl-3-phenylazoformazan by the further action of benzenediazonium chloride.

These reactions are illustrated by the following diagram:

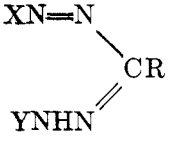


von Pechmann further showed (134) that the two possible isomers of 1(5),3-diphenyl-5(1)-*p*-tolylformazan (XXII, table 16), supposedly obtained from the alternative methods of synthesis (i.e., from benzenediazonium chloride and benzaldehyde *p*-tolylhydrazone in the one case, and from *p*-toluenediazonium chloride and benzaldehyde phenylhydrazone in the other), both reacted with

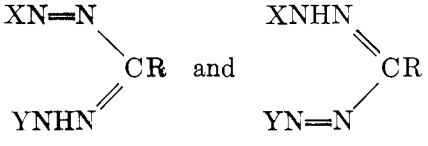
concentrated sulfuric acid in acetic acid to yield the same rearrangement product, 6-methyl-3-phenylbenztriazine.



It was also shown (139) that formazans of the type

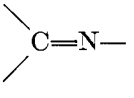


(such as XXII) give all the four products, on reduction with zinc and dilute sulfuric acid, which would be expected from a mixture of

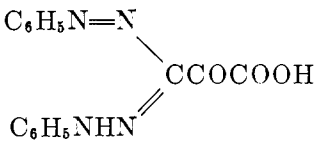


These products are XNHNHCOR and $YNHNH_2$ from the former, and $XNHNH_2$ and YNHNHCOR from the latter. These results contrast with those just described for the purified individual acetyl compounds (XXIa and XXIb), in which the tautomerism is fixed.

Nevertheless, several workers claimed to have isolated different compounds when the aryl substituents in the diazonium salt and the phenylhydrazone were interchanged. Bamberger and his coworkers (18, 19, 97) showed that the action of benzenediazonium chloride on pyruvic acid in alkaline solution gave formazylglyoxylic acid (XXIII, table 13). This formed red needles which were converted into an isomeric yellow form by treatment with zinc chloride and acetic anhydride. Cold concentrated sulfuric acid caused a reversion to the red form. The authors suggested that the forms were *cis–trans* isomers about the

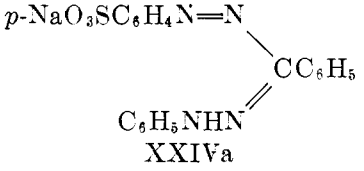


bond.

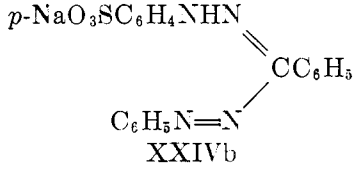


XXIII

Fichter and Schiess (66) claimed that the formazans (XXIVa and XXIVb, table 16) prepared from diazotized sulfanilic acid and benzaldehyde phenylhydrazone, and from diazotized aniline and benzaldehyde *p*-sulfophenylhydrazone[2], respectively, were different. This conclusion was, however, based merely on differences in the decomposition points of the sodium salts (the free formazans were not isolated) and the apparently different colors which they gave in the dyeing of silk and wool. Both compounds were shown to give the same product, β-benzoyl-4-sulfophenylhydrazide, upon reduction.



XXIVa



XXIVb

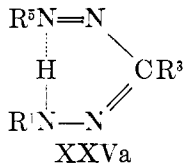
Pairs of formazans containing α- or β-naphthyl groups attached to either terminal nitrogen atom were shown to possess more marked differences in properties.

Busch and Schmidt (46) also concluded, after careful experiments, that the formazan resulting from the condensation of benzenediazonium chloride with benzaldehyde *p*-bromophenylhydrazone was different from that obtained from *p*-bromobenzenediazonium chloride and benzaldehyde phenylhydrazone.

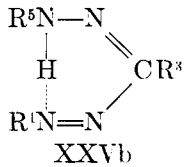
Lapworth (113) prepared optically active formazans (e.g., the *l*-menthyl ester of formazylcarboxylic acid, table 13) with the intention of demonstrating mutarotation as indicative of tautomerism. Unfortunately he was unable to measure the optical rotations.

In 1941 Hunter and Roberts (94) established conclusively that the products of the alternative synthetic routes to a formazan were indistinguishable. The identity of the products was established in several cases by mixed-melting-point determinations both of the formazans themselves and of their copper, cobalt, and nickel complexes. A chelated structure was indicated by the solubility in most organic solvents, the relatively low melting points, and the unimolecular character in solution of the formazans, both in the free state and as metal complexes.

These workers therefore proposed an internally coördinated hydrogen-bond structure, which can exist in two mesomeric forms, XXVa and XXVb. The formazan molecule thus appears to be a resonance hybrid of these forms:



XXVa

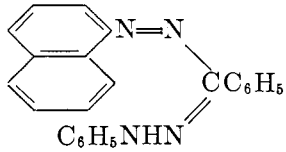


XXVb

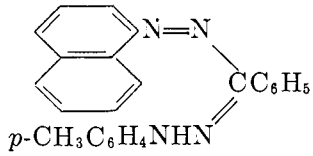
[2] Doubt has recently been cast on the true phenylhydrazone character of this compound.

The original views of von Pechmann were thus confirmed but were given a new theoretical interpretation. Three alleged pairs of isomers previously described in the literature (46, 64, 66) were shown by the same workers to be three single substances.

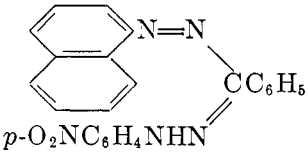
At the same time Kuhn and Jerchel produced a similar theory (106), which was further confirmed by their proof of the identity of Debye–Scherrer x-ray diagrams and absorption spectra of supposed pairs of formazans. These conclusions have been amplified by the modern Italian school of workers; the stannous chloride reduction of the individual members of certain postulated pairs of formazans (149) led to the isolation of the same product from each individual, showing that these individuals were in fact identical. Subsequently, Ragno and Oreste (150) studied various nitro-substituted formazans, whose properties were consistent with the mesomeric theory of formazan structure. They also prepared three pairs of formazans bearing naphthyl groups, and their results showed that the individual members of these pairs (XXVI–XXVIII) (table 16) were not identical. The evidence was principally that of melting points and mixed melting points.



XXVI

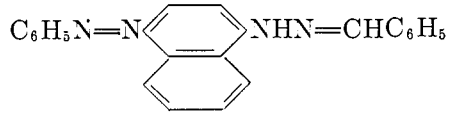


XXVII



XXVIII

These workers put forward the suggestion that although all formazans exist in mesomeric states, the mesomeric equilibrium could be displaced towards one or other of the limiting forms when the extent of the dissymmetry between the two groups attached to the azo and hydrazone chains was appropriate. It was suggested that *o*- and *p*-substituted nitro groups produced a dissymmetry which was particularly effective. This problem has recently been clarified by Hausser, Jerchel, and Kuhn (83), who showed that the product of condensation of benzenediazonium chloride and benzaldehyde 1-naphthylhydrazone was not the formazan XXVI but XXIX, obtained by azo-coupling on to the naphthalene ring.



XXIX

The stannous chloride reduction of XXIX gave 1,4-diaminonaphthalene, and its structure was further established by the absorption spectrum and the inability of the substance to form metallic complexes. No change in the absorption spectrum of XXIX occurred after irradiation, while the spectra of formazans do show such a change (page 370). On the other hand, naphthalene-1-diazonium

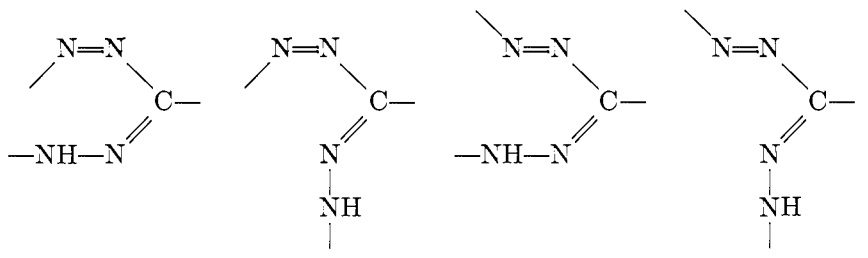
chloride reacted with benzaldehyde phenylhydrazone to give the expected formazan (XXVI), so that the differences in properties of the products of Ragno and Oreste were very simply explained. Other apparently anomalous pairs of formazans described by these workers were explained in a similar manner.

To sum up, therefore, when all three substituent groups in a formazan are alike, only one form can be synthesized. When two are the same, only two isomers can be prepared: one with the odd group attached to carbon and the other with the odd group attached to nitrogen. When all three substituents are different, three isomers can be prepared.

B. STRUCTURAL INTERPRETATION OF COLOR CHANGES IN FORMAZANS

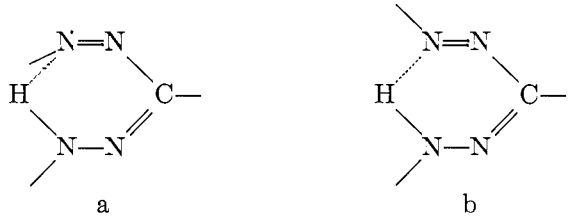
Recently Hausser, Jerchel, and Kuhn (82), in further studies on the structure of formazans, have shown that the problem is complicated by the observation that many formazans, previously thought to be single substances, could, on exposure to visible light, be obtained in red and yellow forms.

The classical spatial arrangement of a formazan molecule allows the existence of four possible structures due to geometrical isomerism about the two double bonds (the possibilities of tautomerism being ignored for the present). These may be depicted diagrammatically as shown below (XXXa–d):



cis-syn XXXa | *cis-anti* XXXb | *trans-syn* XXXc | *trans-anti* XXXd

If the more recently postulated chelate structure involving hydrogen bonding is accepted for formazans, the *cis-syn* (XXXa) and *trans-syn* (XXXc) forms (i.e., those with the *cis* configuration about the C=N— bond) must be depicted as in XXXI.



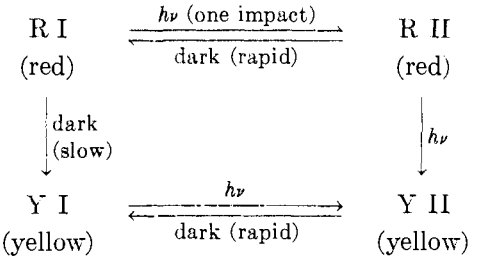
a b

XXXI

It might appear that this was not possible in XXXIa for steric reasons. The use of models shows, however, that a *cis-syn* structure is consistent with hydrogen bonding because of the non-planar arrangement of the molecule.[3] In the *cis-anti* (XXXb) and *trans-anti* (XXXd) forms of XXX (with a *trans* configuration about the $\diagdown C{=}N{-}\diagup$ bond), on the other hand, hydrogen bonding does not apprear to be spatially possible.

Kuhn and his coworkers therefore suggested two possible explanations for the fact that some formazans can be converted from a red form to a yellow form by irradiation with visible light and that the reverse reaction occurs in darkness. Hausser (80) measured the quantum yields of the red $\rightleftharpoons$ yellow change in triphenylformazan irradiated by light of wave length 490 mμ. His results showed that this change involved a photochemical intermediate which was reconverted to the red form after a half-life of 17 sec.

Such measurements showed a cycle of events involving two yellow and two red forms. The red form R I, stable in the solid state, is converted to a short-life red form R II, which is converted by another quantum of light to the yellow form Y II. This, too, is of short life and rapidly passes in darkness to the more stable yellow form Y I. This reverts to the R I form slowly in darkness, but catalysts influence the change strongly. The absorption spectra of the short-life intermediates R II and Y II show small differences from those of R I and Y I, respectively. The diagram summarizes the changes observed:

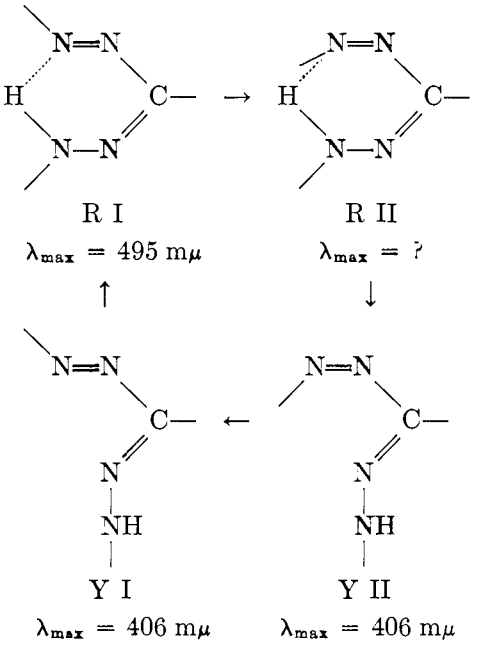


Kuhn and Weitz (109) then discussed the precise allocation of these four experimentally observed forms to the theoretical structures mentioned above (XXXa–d). They assumed that the observed changes were due to interconversions of the geometrical isomers, accompanied by opening or closing of the chelated hydrogen bridge. By analogy with the changes in the light absorption of the different geometrical isomers of certain azo compounds, they assigned the

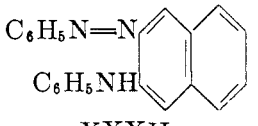
[3] If the chelated ring forms a mesomeric system, resonance theory requires the ring to have a planar structure, and the forms XXXIa and XXXIb are indistinguishable. This makes it difficult to explain the structural difference between the two red forms R I and R II observed experimentally. It is also curious that, according to the theoretical interpretation of Hausser, Jerchel, and Kuhn, the *open-chain*, highly conjugated structures (XXXb and XXXd) are *yellow*, and the mesomeric forms (XXXa and XXXc) are the intensely *red* forms. Present views of the relation between color and structure in organic compounds would expect the reverse to be the case.

stable red form R I to the *trans-syn* form (XXXc) (page 369), and the unstable red form R II to the *cis-syn* form (XXXa). Thus the first change on illumination (R I → R II) involves a change of configuration about the —N=N— system from *trans* to *cis* without rupturing the hydrogen bridge. In the change to yellow (R II → Y II), the chelation is destroyed (together with the resonance in the molecule) when the —C=N— bond assumes a *trans* or *anti* configuration. Yellow form Y II is the *cis-anti* form (XXXb), and the more stable yellow form Y I is the *trans-anti* form (XXXd). The rapid dark reactions are *cis–trans* conversions and the slow dark reaction is an *anti–syn* change.

The cyclic process in light can therefore be rewritten in terms of the isomeric structures:



Indirect confirmation of these theories is found in the classical *cis–trans* conversion in azobenzene and in the same type of conversion in 1-benzeneazo-2-phenylaminonaphthalene (XXXII), which Kuhn and Weitz observed upon its illumination in benzene. XXXII underwent a change very like that of R I → R II above.

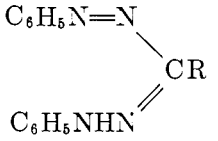


XXXII

The spectra of the yellow forms in which the hydrogen bridge has disappeared are analogous to those of ordinary *cis–trans* isomers of azo compounds. It ap-

pears that the *cis–trans* change about the azo group occurs more readily under the influence of light than the *syn–anti* change about the $\diagdown\!\!\diagup C{=}N-$ bond. This one would expect *a priori*; in fact, the *syn–anti* change only takes place when the azo group has already been converted to the *cis* configuration.

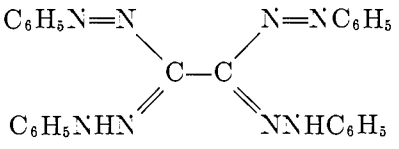
Hausser, Jerchel, and Kuhn showed that the occurrence of two forms of a formazan, red and yellow, was, if not universal, at least of wide occurrence. In the formazans of formula XXXIII (table 10), when R = methyl, the solid is yellow and forms a yellow solution in methanol. When it is dissolved in benzene and kept in the dark, an equilibrium mixture of red and yellow forms is obtained. This was analyzed by measuring the absorption spectra. When R = *n*-heptyl, the solid is red, but gives a solution of the almost pure yellow form in ethanol, and an almost pure red form in benzene.



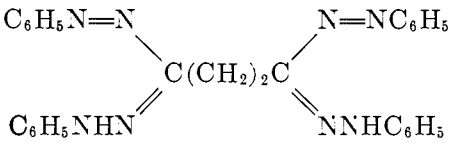
XXXIII

The prediction that in an intermediate case, the two forms might be isolated in a pure state from different solvents was realized in the case where R = ethyl. This formazan was obtained as orange needles, m.p. 102–103°C., from methanol, and as red needles, m.p. 73–75°C., from benzene. No exact assignation of structure to these two forms was attempted. When R = aryl in XXXIII (table 16), the solid is red and the solution in both ethanol and benzene is red, but irradiation gives a pure yellow form. This yellow form is stable only in the solvated state and spontaneously reverts to the red form upon the removal of solvent. When redissolved, this solid shows only the red absorption spectrum. The change to yellow on irradiation occurs more slowly in ethanol. An attempt to stabilize the yellow form by the formation of mixed crystals with diphenylmethane was unsuccessful. The activation energy required for the isomeric change must be exceedingly low; the successful isolation of the solid yellow form of the *C*-ethylformazan indicates the existence of a higher energy peak in this case. This behavior appears to be paralleled in the 6-hydroxyalkylformazans; 1,5-di-*o*-chlorophenyl-3-(2′-hydroxyethyl)formazan is a red solid, which gives a stable red solution in benzene but whose ethanolic solution is rapidly changed from red to yellow by the action of sunlight. The *O*-acetyl derivative does not show this transformation. 1,5-Di-*o*-chlorophenyl-3-(2′-hydroxypropyl)formazan is yellow, both as a solid and in solution (60a).

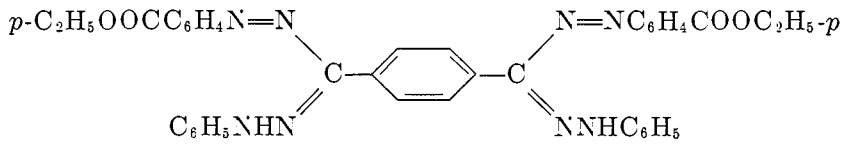
In the case of the diformazan XXXIV (table 20) and several others (163a), only the red form was detected, but the two forms were clearly observed in XXXV and XXXVI.



XXXIV



XXXV



XXXVI

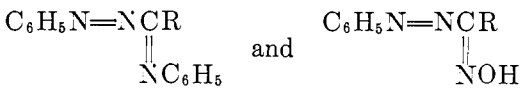
In summing up, it now seems safe to say that, after many years of controversy, the general problem of isomerism in foramazans has been clarified: only one form of the normal stable solid formazan is known, even when the two substituents attached to nitrogen are dissimilar; in solution, the absence or presence of light can give rise to two forms of considerable stability, one with a hydrogen bridge and one without. In addition, two transient forms may occur during the transformation.

The effect of ultraviolet light on formazans is quite different from that of visible light. In the case of triphenylformazan autoxidation occurs, leading to the formation of the diphenylenetetrazolium salt (CX, page 423), a compound which is also produced by the action of ultraviolet light on triphenyltetrazolium chloride (80).

C. ABSORPTION SPECTRA OF FORMAZANS

Wave lengths of maximum absorption of formazans which have been reported in the literature are listed in table 1. Characteristic absorption curves for formazans and tetrazolium salts are shown in figure 1 (48a). Most formazans absorb fairly intensively at wave lengths in the red and violet bands; this gives rise to their characteristic intense colors. They also absorb strongly in the ultraviolet at about 250–350 mμ. The replacement of *C*-alkyl by *C*-aryl groups appears to have little effect on the absorption. The mercapto compounds, which are intensely purple or blue-black, apparently show absorption maxima at about 620–650 mμ (92), although the technique of measurement was not described.

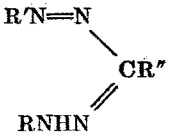
Grammaticakis (76) has described the visible absorption of various azo compounds and has shown that those of the types



give absorption curves typical of simple azo compounds (e.g., benzeneazomethane), whereas formazans of the type of XXXIII (page 372), where R = H, CH_3, C_6H_5, and —N=NC_6H_5, have a distinctive curve, which is approximately the same in all the cases. He did not quote the λ_{max} values for these compounds.

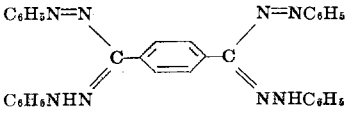
TABLE 1

Absorption spectra of formazans

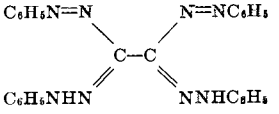


R	R'	R''	Form*	Absorption Maxima†						Solvent‡	References
				λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}		
C_6H_5	C_6H_5	H	l	260	13,000	285	7,400	420	31,000	A	(82)
C_6H_5	C_6H_5	CH_3	l	260	17,000	410	28,500			A	(82)
			d	295	9,200	410	13,000	480	9,200	B	(82)
C_6H_5	C_6H_5	C_2H_5	l	255	17,000	410	25,200			A	(82)
			d	305	9,200			470	13,900	B	(82)
C_6H_5	C_6H_5	n-C_8H_{18}		250	15,600	420	18,300			A	(106)
C_6H_5	C_6H_5	n-C_7H_{15}	l	250	16,000	295	7,400	420	22,500	A	(82)
			d	295	11,700			485	14,800	B	(82)
C_6H_5	4-$O_2NC_6H_4$	n-$C_{11}H_{23}$				427	26,000				(106)
C_6H_5	C_6H_5	OH		234	14,100	285	10,000				(77)
C_6H_5	C_6H_5	SH				440	12,600	590	20,000		(77)
						462		635	32,500	C	(96, 129)
2,4-$(CH_3)_2C_6H_3$	2,4-$(CH_3)_2C_6H_3$	SH				460	11,500	630	48,000		(129)
4-BrC_6H_4	4-BrC_6H_4	SH				452		629	30,600	C	(96)
5-Cl-2-HOC_6H_3	5-Cl-2-HOC_6H_3	CN						504		A	(201)
5-Cl-2-HOC_6H_3	5-Cl-2-HOC_6H_3	$-COC_6H_4$-2-COOH						530		A	(201)
C_6H_5	C_6H_5	C_6H_5	l	290	10,000	405	30,900			B	(82)
			d	300	21,700			500	16,100	B	(82)
			d	260	17,800	295	23,800	485	17,000	A	(82)
C_6H_5	α-$C_{10}H_7$	C_6H_5	l	290	16,900	430	21,300			B	(82)
			d	275	19,600			520	16,100	B	(82)
C_6H_5	β-$C_{10}H_7$	C_6H_5	l	250	23,500	290	17,400	400	28,300	E	(82)
			d	270	24,800	305	28,300	505	19,100	E	(82)
C_6H_5	4-$C_6H_5C_6H_4$	C_6H_5	l	280	16,900	295	17,800	420	36,500	B	(82)
			d	280	24,300	310	31,700	510	21,300	B	(82)
			d	240	22,600	310	37,000	510	22,200	A	(82)

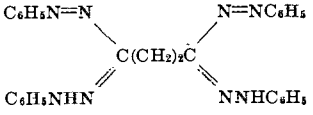
C_6H_5	C_6H_5	4-$C_6H_5C_6H_4$	1			410	29,100			B	(82)
			d	320	33,000			500	14,800	B	(82)
			d	255	21,700	320	35,200	490	15,200	A	(82)
				265	20,800						
4-$C_6H_5C_6H_4$	4-$C_6H_5C_6H_4$	C_6H_5	1			435	37,000			B	(82)
			d	320	29,600			550	23,000	B	(82)
			d	255	17,400	320	30,000	530	?	A	(82)
				280	19,600						
C_6H_5	4-ClC_6H_4	C_6H_5	1	290	13,500	410	30,000			B	(82)
			d	305	23,500			490	16,100	B	(82)
			d	300	25,600			485	16,900	A	(82)
C_6H_5	4-IC_6H_4	C_6H_6						500	17,500	D	(70)
4-IC_6H_4	4-IC_6H_4	C_6H_5						510	16,000	D	(70)
α-$C_{10}H_7$	4-$O_2NC_6H_4$	C_6H_5	1	290	18,700	365	12,600	438	17,400	B	(83)
			d			365	14,800	505	21,700	B	(83)
4-$O_2NC_6H_4$	4-IC_6H_4	C_6H_5						500	11,000	D	(70)



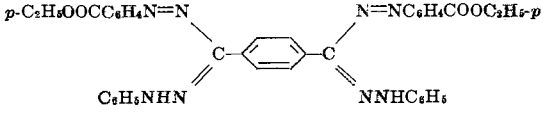
						330		520	18,500		(82, 100)



				260	22,200	285	24,000	470	32,600	A	(82, 100)
				285	20,900	305	20,000	485	37,400	B	(82)

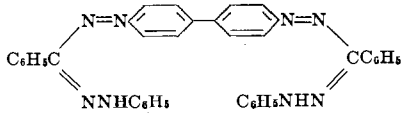
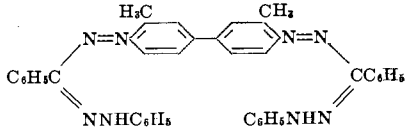
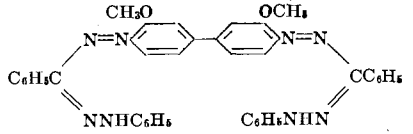
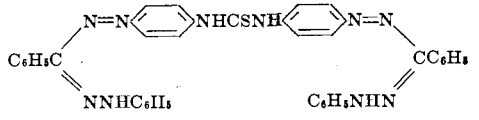
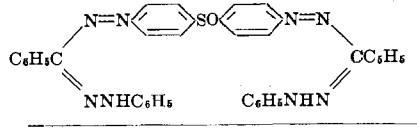


				255	30,800	410	31,700			A	(82)
				295	14,800			470	25,200	B	(82, 100)



			1	290	28,200	410	41,700			B	(82, 100)
			d	325	35,600			510	22,600	B	(82, 100)

TABLE 1—*Concluded*

	Form*	Absorption Maxima†						Solvent‡	References
		λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}		
	1					555	10,600		(163a)
	d					572	18,500		(163a)
						587	14,400		(163a)
						606	10,400		(163a)
						512	16,100		(163a)
						495	13,100		(163a)

*1 = form stable in light; d = form stable in darkness.

† Many of the figures are estimated from published graphs.

‡ A = ethanol, B = benzene, C = carbon tetrachloride, D = dioxane, E = cyclohexane.

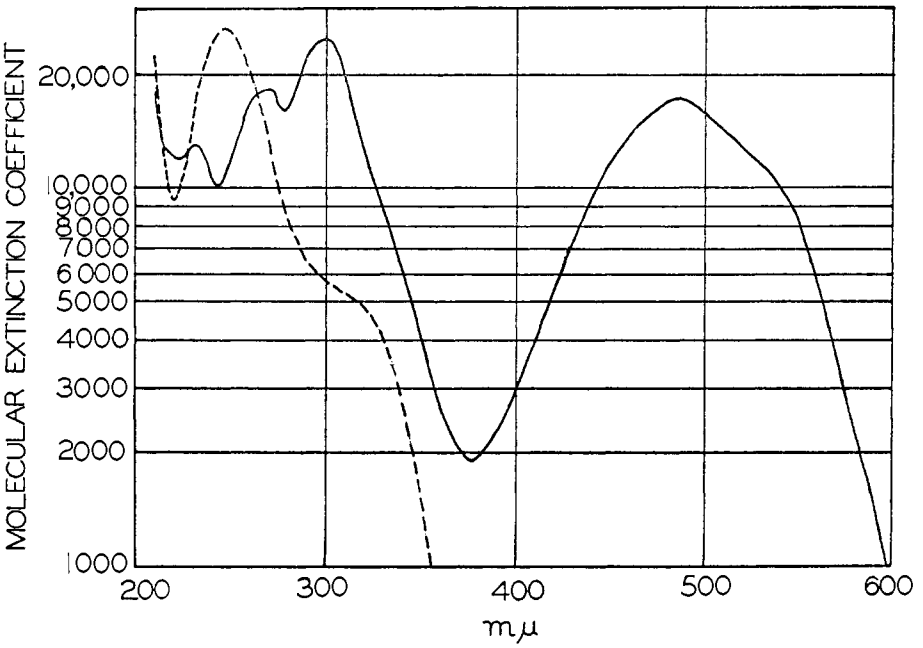
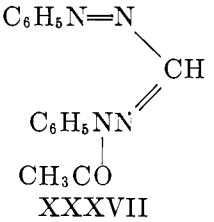


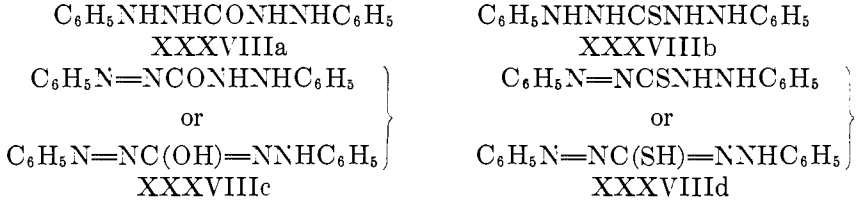
FIG. 1. Absorption curves of 1,3,5-triphenylformazan and of 2,3,5-triphenyltetrazolium bromide. ——, formazan in ethanol; - - -, tetrazolium salt in water.

The acylated formazan (XXXVII) was shown to have a curve like that of the above azo compounds, presumably because the chelation and mesomerism of the formazan molecule have been destroyed. The same author (77) has also thrown light on the problem of tautomerism in *C*-hydroxy- and *C*-mercaptoformazans.



XXXVII

Curves of the middle and the long ultraviolet for diphenylcarbazide (XXXVIIIa) and diphenylthiocarbazide (XXXVIIIb) resemble that of phenylsemicarbazide, but whereas diphenylcarbazone (or 3-hydroxy-1,5-diphenylformazan, table 12) (XXXVIIIc) gives a curve like that of an azo compound, the curve of its sulfur analog (XXXVIIId) is that of a formazan.



XXXVIIIa

XXXVIIIb

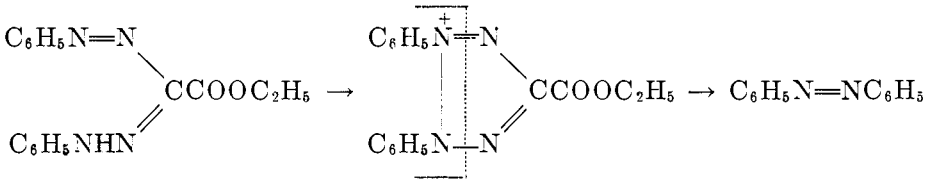
XXXVIIIc

XXXVIIId

These results do not imply that the alternative form in each case is entirely absent.

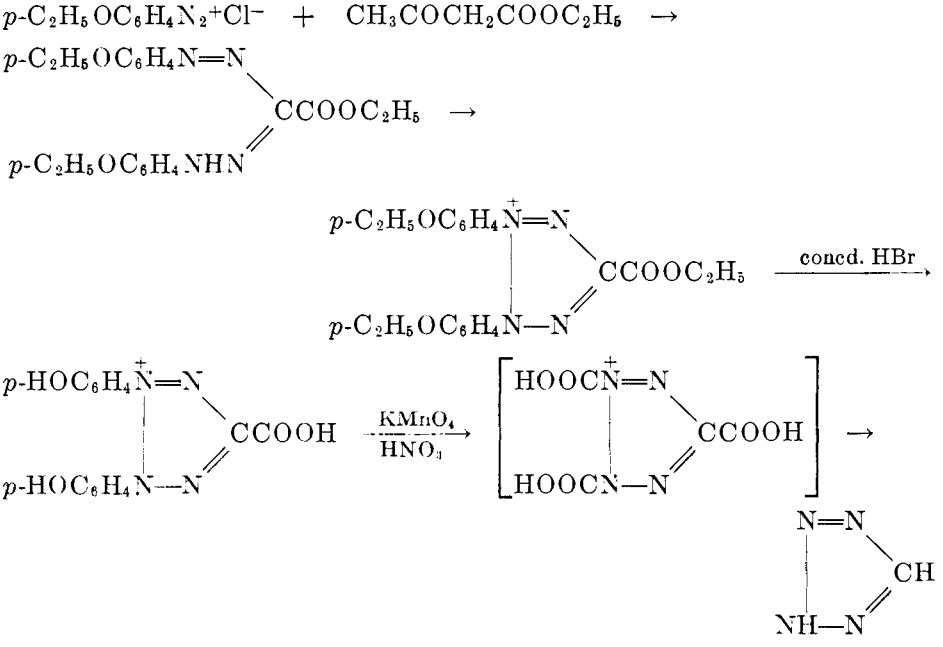
D. MOLECULAR STRUCTURE OF TETRAZOLIUM SALTS

The theory that tetrazolium salts were quaternary derivatives of tetrazole was first proposed by von Pechmann (140). His suggestion was based on the fact that these compounds are formed by the oxidation of formazans, and on their powerfully basic, highly ionized character. This view was supported by the oxidation of 1,5-diphenyl-3-ethoxycarbonylformazan (XXXIX) to the tetrazolium salt (2,3-diphenyl-5-ethoxycarbonyltetrazolium chloride) and the dry distillation of the latter to yield azobenzene.



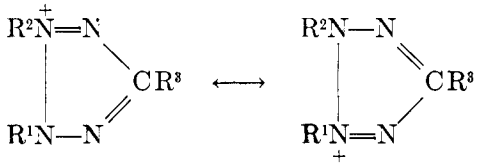
XXXIX

Wedekind and von Pechmann (141) confirmed this theory by the preparation of a 5-carboxy-2,3-diphenyltetrazolium salt which was substituted on the phenyl groups in such a way that they could be readily oxidized by acid permanganate to tetrazoletricarboxylic acid, which was spontaneously decarboxylated to tetrazole. The presence of hydroxy groups fulfilled this requirement and the reactions studied were:



When the same procedure was repeated with a tetrazolium salt containing but one hydroxyphenyl group, the expected diphenyltetrazole was obtained; only one of the possible isomeric forms of this compound was isolated (180, 183).

Wedekind failed in an attempt (188) to resolve a tetrazolium molecule by crystallization of 2,3,5-triphenyltetrazolium *d*-camphorsulfonate and of the *d*-bromocamphorsulfonate. Modern theories of mesomerism suggest that the tetrazolium molecule is a resonance hybrid of the possible extreme forms (a) and (b):



(a) (b)

In asymmetrical compounds in which R^1 and R^2 are different, only one tetrazolium salt is predicted and this is confirmed in practice.

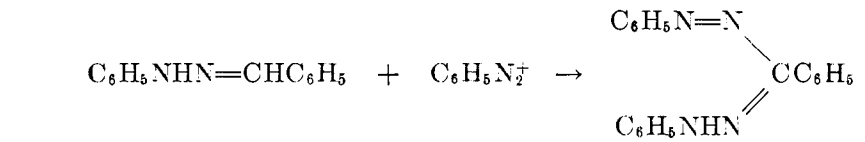
VI. Synthesis of Formazans

The methods by which formazans have been prepared have been grouped in broad classes, which are discussed below. This classification has been used in tables 9–26.

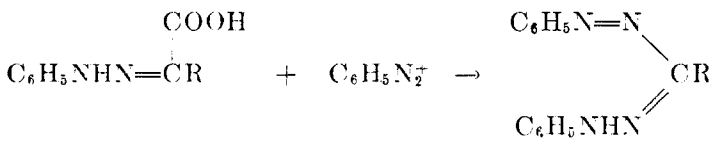
A. FROM DIAZONIUM SALTS

1. By the action of diazonium salts on arylhydrazones (method A1)

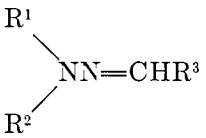
The coupling of diazonium salts with aldehyde or glyoxylic acid arylhydrazones is carried out at an alkaline pH, usually in the presence of caustic alkalies, pyridine, or sodium acetate. The majority of known formazans have been prepared by this method, which is the standard one for the triaryl type. In a few instances, analogous reactions have been carried out with mesoxalic ester phenylhydrazones. The reaction is expressed by the equations:



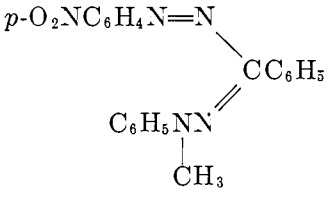
or



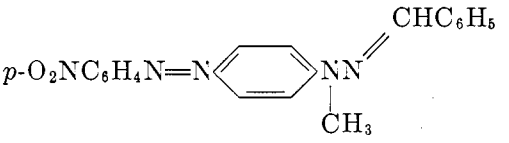
The mechanism of the reaction has been studied by Busch and his coworkers (43, 44, 45, 46). It had been shown by von Pechmann (134) that secondary phenylhydrazones of the type



do not react with diazonium salts to give formazans, and it appeared therefore that the imide hydrogen atom played an essential part in the reaction. On the other hand, Bamberger had claimed that *p*-nitrobenzenediazonium chloride reacted with benzaldehyde methylphenylhydrazone to give 5-methyl-1-*p*-nitrophenyl-3,5-diphenylformazan (XL).



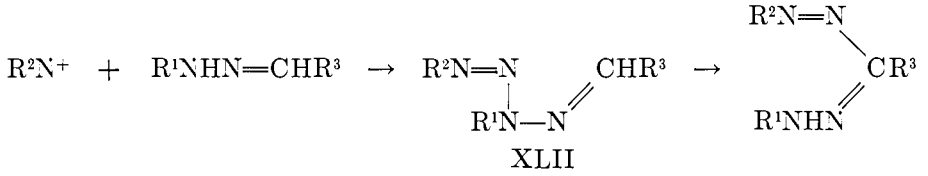
XL



XLI

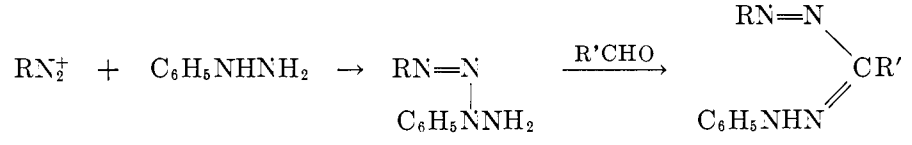
Busch and Schmidt (45) showed that Bamberger's product was actually the azo compound (XLI), the structure of which was confirmed by reduction and hydrolysis. The same behavior was observed in the case of benzaldehyde phenylbenzylhydrazone. Benzaldehyde dibenzylhydrazone gave neither azo compound nor formazan, since there was no aromatic nucleus activated towards azo coupling available.

Busch and Pfeiffer (43) therefore postulated that the first reaction in the course of formazan formation was the coupling of a diazonium ion to the imide nitrogen with displacement of hydrogen to give XLII, which then rearranged to the formazan.



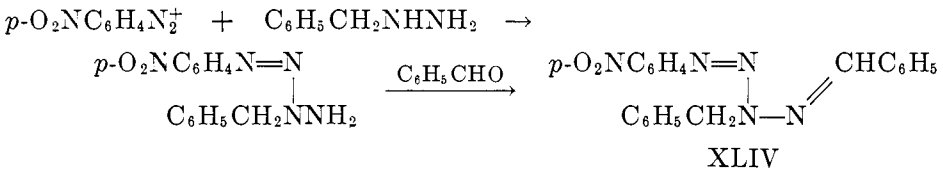
XLII

It was already known that diazonium salts couple on the α-nitrogen atom of primary phenylhydrazines (202) to give α-diazohydrazines (XLIII).



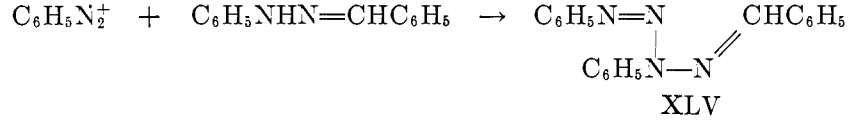
XLIII

The latter compounds react with aromatic aldehydes to give formazans. When the hydrazines are non-aromatic, rearrangement to the formazan does not take place. Benzylhydrazine, for example, gives the end-product XLIV.



XLIV

XLIV was also obtained by coupling *p*-nitrobenzenediazonium chloride with benzaldehyde benzylhydrazone. That this type of intermediate is present in the formation of formazans was demonstrated by Busch and his coworkers when they isolated the unstable compound XLV from the reaction of benzenediazonium chloride with benzaldehyde phenylhydrazone in ethanol in the presence of sodium acetate at 0°C. This rapidly rearranged to the formazan in solution with a change in color from yellow to red.

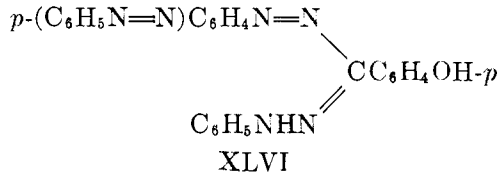


XLV

Busch and Schmidt (46) summarized as follows the action of diazonium compounds on different types of hydrazones: (*1*) Arylhydrazones of aldehydes react with diazonium hydroxides or with diazotates to give formazans; with diazonium salts they yield azohydrazones. (*2*) Aldehyde secondary hydrazones react with difficulty with diazonium salts, and with great difficulty with diazotates, to give azohydrazones. No product is formed if the aryl group in the hydrazone is not sufficiently activated for azo coupling. (*3*) Ketone hydrazones couple with diazonium salts to give azohydrazones under the same conditions as in 2 (44).

Views about the exact coupling mechanism of diazonium compounds remain controversial to the present day. A recent paper (163) denies Busch's mechanism of formazan formation (page 401; see also page 391), but another report confirms it and has established that the tetrazene–formazan rearrangement is probably intramolecular (79a). In this work, the 1-benzal-2,4-diphenyltetrazene (XLV) was prepared in 70 per cent yield by carrying out the usual diazonium salt–phenylhydrazone condensation at a pH of 6–8. It was shown that this tetrazene is dissociated by acids into the starting materials. The speed of the tetrazene–formazan rearrangement was greatest in pyridine and least in alcohol of the media studied. When the rearrangement of XLV took place in the presence of *p*-nitrobenzaldehyde phenylhydrazone only triphenylformazan was isolated, and in the presence of R salt no azo compound was formed. The possible reactions between diazonium compounds and hydrazones may frequently occur simultaneously. Fichter and Fröhlich (64) showed that the treatment of a mixture of α-naphthol and benzaldehyde phenylhydrazone with a diazonium salt led to the formation of a 50 per cent yield of formazan and a 20 per cent yield of hy-

droxynaphthylazo compound. When β-naphthol, which has greater readiness to undergo azo-coupling, was used, no formazan was obtained. In the coupling of benzenediazonium chloride with salicylaldehyde phenylhydrazone (where either reaction might be expected), only the formazan was isolated.



XLVI

Recent work has shown (117) that formazans of the type of XLVI (table 18) are obtained when *p*-acetoxybenzaldehyde phenylhydrazone is coupled with diazotized 4-aminoazobenzene in pyridine. The formazan acetate is isolated and can readily be hydrolyzed to XLVI. No formazan can be isolated if *p*-hydroxybenzaldehyde phenylhydrazone is used, and this is probably due to the preferential azo-coupling on to the aromatic ring activated by the presence of a hydroxyl group.

Similarly, the preliminary protection of amino groups, whether in the diazonium salt or in the phenylhydrazone, is necessary before the coupling reaction is attempted (2).

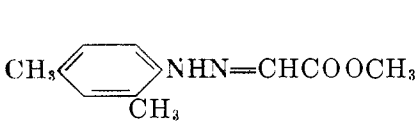
The general experimental procedure for this method of preparing formazans has been reviewed by von Pechmann (133). The only notable modern modification in technique has been the use of pyridine as a coupling medium, apparently first introduced by Kuhn and Jerchel (106) and used frequently since. This appears to have been based on experience of the value of pyridine as a promoter of azo-coupling in the dyestuffs industry. Sometimes pyridine and sodium hydroxide or sodium acetate have been used in conjunction. Other bases, such as triethylamine, also promote formazan formation.

A number of failures with the procedure have been reported; in many cases, a deep red or purple color appears in the solution, and it is not clear whether the yield is negligible, or whether the formazan is not present in a sufficiently high yield to enable it to be crystallized from the complex reaction mixture. Unless a formazan does crystallize directly from the reaction mixture, or can be separated by a simple process of dilution with water or of acidification, the reactivity and consequent instability of the formazan molecule can make isolation difficult. Many of the more complex formazans have a low solubility in most organic solvents and can therefore be isolated even when formed in quite small yields. In the case of those formazans rendered soluble in ionizing solvents by the presence of quaternary ammonium groups (126), isolation is difficult, the yields are poor, and modified techniques have to be used. Wedekind's failure to introduce hydroxy groups into formazans (183, 191) may be attributable to influences already mentioned[4] and to the fact that the *p*-benzenediazo oxide (diazotized *p*-aminophenol) which he tried to condense with *p*-nitrobenzaldehyde phenyl-

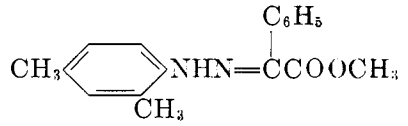
[4] See the discussion about XLVI on page 382.

hydrazone is well known to be a "weak" coupling agent. His failure to introduce sulfonic acid groups into formazans may have been due to difficulties of isolation. Fichter and Schiess (66), using the "inverted-coupling" technique, succeeded in isolating several sulfonated formazans as their sodium salts. The free compounds were not isolated and the products were not well characterized. These workers failed to obtain formazans from *o*-chlorobenzaldehyde phenylhydrazone, 3,4-dihydroxybenzaldehyde phenylhydrazone, and nitroformaldehyde α-naphthylhydrazone.

Busch and von Beust (40) studied the behavior of arylhydrazones known to exist in two modifications; they showed that the so-called α- and β-forms of glyoxylic acid *as*-xylylhydrazone both reacted in the expected manner with diazonium salts to give the one formazan, 1-aryl-3-carboxy-5-*as*-xylylformazan. The α-form of methyl glyoxylate *as*-xylylhydrazone (XLVIIa) similarly gives the corresponding methyl ester of this formazan, but the β-form of XLVIIa yields the *as*-xylylhydrazone of methyl benzoylformate (XLVIIb).



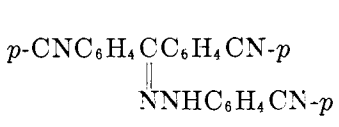
XLVIIa



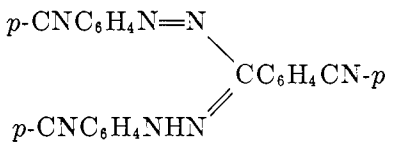
XLVIIb

The α- and β-forms of glyoxylic acid phenylhydrazone showed similar differences of behavior on treatment with diazonium salts. These forms have been regarded as *cis–trans* isomers, and the β-form has been identified tentatively with the *trans*-isomer.

Failures have also been reported recently (2) in attempts to prepare formazans using *p*-dimethylaminobenzenediazonium chloride (weak coupling power) and *p*-hexylbenzenediazonium chloride; in the latter case the physical characteristics of the product appeared to defeat purification. Cinnamaldehyde phenylhydrazone did not appear to couple in the desired manner, and some complex amines bearing phenylazo and styryl groups as substituents failed to give formazans. Only minute traces of the formazan (XLVIIIa) were isolated from the addition of *p*-cyanobenzenediazonium chloride to *p*-cyanobenzaldehyde *p*-cyanophenylhydrazone. The main product was 4,4′-dicyanobenzophenone *p*-cyanophenylhydrazone (XLVIII), formed by the elimination of a molecule of nitrogen.



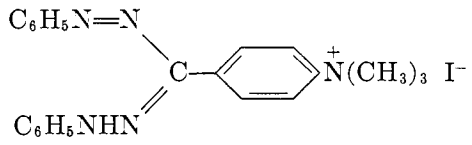
XLVIII



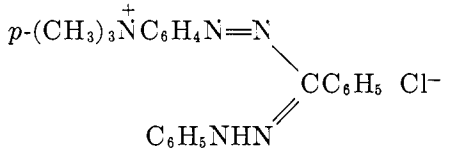
XLVIIIa

Little definite evidence is available concerning the influence of aromatic substituents in the two components involved in the formation of formazans. The effect of the activation of benzene rings towards the alternative azo-coupling

reaction shown by groups such as dialkylamino and hydroxyl has already been mentioned (page 381). The available evidence suggests that, in general, an electrophilic group in the meta or para position of the phenylhydrazine moiety reduces the extent of formazan formation. Such a group in the aromatic aldehyde has a similar, but less pronounced, effect. It seems probable that this is the explanation of some of the failures experienced in attempts to prepare formazans containing quaternary ammonium groups. The two simple compounds XLIX and L (table 16) of this class were isolated in moderate yields, and analogs of XLIX could be prepared from *p*-acetamidoaniline, *o*-benzyloxyaniline, *p*-dimethylaminoaniline methochloride, and 4-(4′-hydroxyphenyl)azoaniline, by diazotization and coupling with *p*-dimethylaminobenzaldehyde phenylhydrazone methiodide. Attempts to isolate a formazan failed with *p*-toluidine, *o*- and *p*-anisidine, *m*- and *p*-nitroaniline, *p*-aminophenol, α- and β-naphthylamine, and *p*-benzyloxyaniline. Similarly, attempts to condense benzenediazonium chloride with the *p*-nitro- and *m*- and *p*-chlorophenylhydrazones of quaternized *p*-dimethylaminobenzaldehyde were unsuccessful (126).

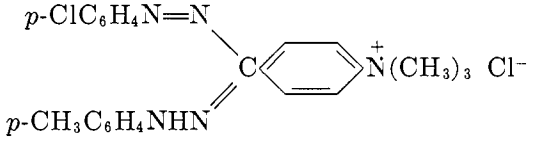


XLIX



L

A further measure of support for the hypothesis advanced above is provided by the fact that when *p*-dimethylaminobenzaldehyde phenylhydrazone methiodide is replaced by the *p*-tolylhydrazone methiodide, the formazan LI (table 16) can then be isolated in moderate yield.



LI

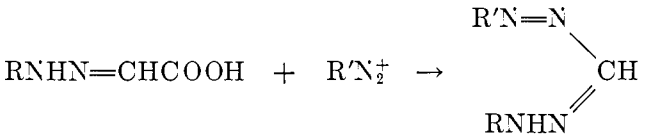
This suggests that the *p*-methyl substituent infuses into the system reactivity which is lacking in the simple phenylhydrazone.

Another factor of apparent significance in formazan formation is that larger substituents in the ortho position of the aromatic aldehyde ring may inhibit formazan formation for steric reasons; attempts to prepare formazans from 2-chlorobenzaldehyde phenylhydrazone (64) and 2,4-dichlorobenzaldehyde phenylhydrazone (127) failed. This factor may not be of consequence in the diazonium salt, since 2,4,6-tribromoaniline has been successfully diazotized and condensed with *p*-bromobenzaldehyde phenylhydrazone (2).

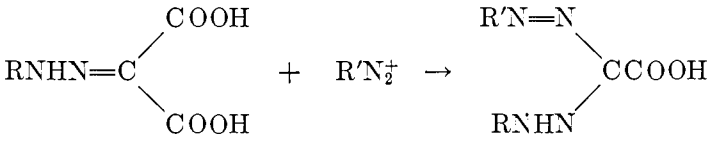
Numerous formazans have been prepared from heterocyclic hydrazones of various aldehydes in recent years, and no special limitations of this reaction have been reported (see table 19).

Bisformazans are no more difficult to prepare than simple formazans, the formazyl nuclei being linked either by aliphatic chains or by aromatic rings. A large number of such compounds have recently been prepared because of their intense colors (see tables 20 and 21). Attempts to extend the aliphatic chain beyond six carbon atoms probably failed because of the instability of the bis-phenylhydrazones of higher dialdehydes (2, 117).

In a variation of the general method of formazan formation, glyoxylic acid phenylhydrazones can be coupled successfully with diazonium salts, under similar conditions, with loss of carbon dioxide (5, 132, 197).



Mesoxalic ester phenylhydrazones undergo an analogous reaction if they are first hydrolyzed to the acid (132). This may be accomplished *in situ* by the use of a strongly alkaline medium.



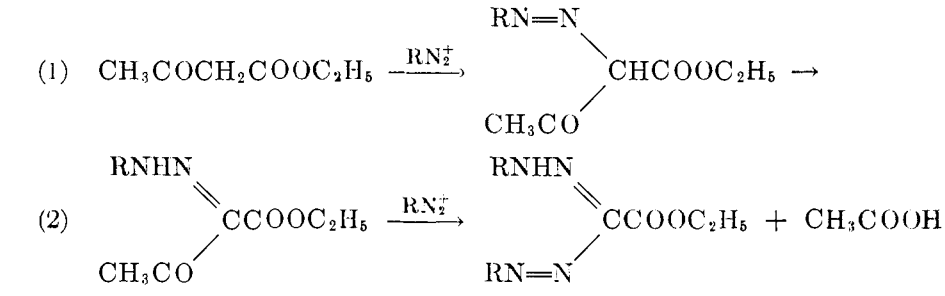
The monophenylhydrazones of aldehydo sugars couple normally with benzenediazonium chloride in an ethanol–pyridine mixture (204). In this way, the glucose, galactose, and mannose molecules have been linked to the formazan system (table 15). The preparation of the glucose compound was complicated by the observation that only glucose β-phenylhydrazone appeared to couple to give a formazan (in 32 per cent yield), whereas the α-phenylhydrazone, which has a cyclic structure, gave no formazan (204). The pentaacetylated formazan was prepared in the case of galactose, both by direct acetylation with acetic anhydride in pyridine and by coupling of benzenediazonium chloride with penta-acetylgalactose phenylhydrazone (207). An attempt to condense a diazonium salt directly on to galactose itself was unsuccessful (207).

2. By the action of diazonium salts on compounds containing active methylene or methine groups (method A2)

(a) By the action of diazonium salts on the broad class of compounds which contain active methylene groups

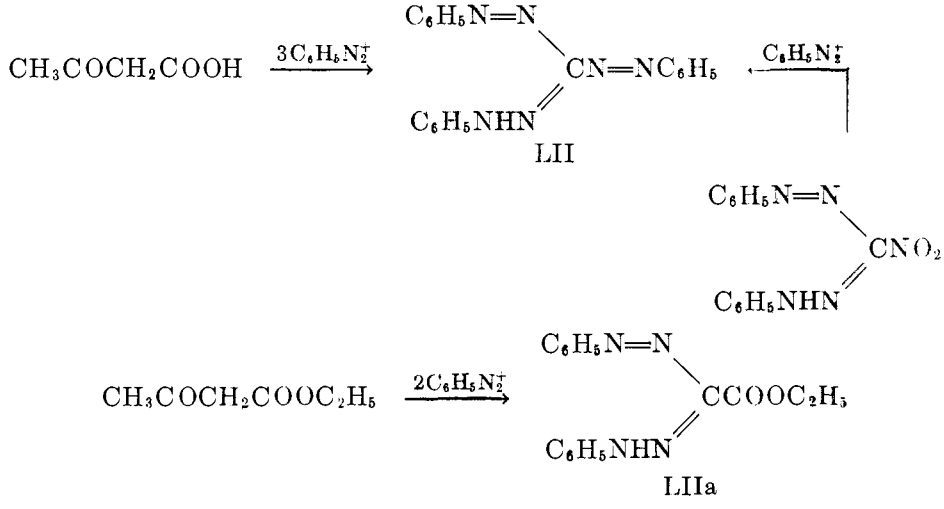
It has long been known that diazonium salts may be coupled with a variety of compounds containing active methylene groups, the activation of which is usually due to a carboxyl or nitro group on the α-carbon atom. The familiar Japp–Klingemann reaction, carried out in nearly neutral solution, embodies the substitution of one diazonium ion in the methylene group to give the azo compound, which rearranges to a phenylhydrazone. When the solution is more al-

kaline and two molecules of diazonium salt are used, a second diazonium ion may couple to the phenylhydrazone molecule first formed. This depends on the ability of the diazonium ion to expel other groups.

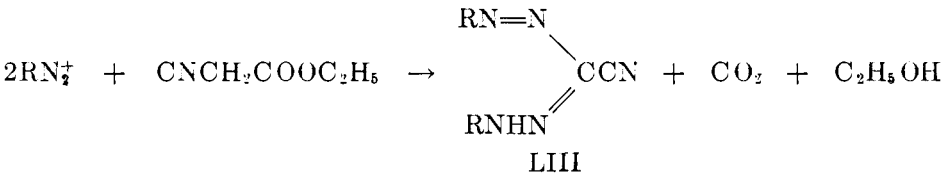


The various types of active methylene compounds which yield formazans are listed in table 2.

von Pechmann (132) recognized the ability of diazonium ions to replace hydrogen, acetyl, and carboxyl groups and further asserted that ethoxycarbonyl and benzoyl groups behaved in a like manner. This latter view was contested by Bamberger (6), who showed that acetoacetic *acid* gave "formazylazobenzene" (LII) (table 18) in which acetyl, carboxyl, and hydrogen had all been replaced, but that ethyl acetoacetate gave almost pure 1,5-diphenylformazan-3-carboxylic ester (LIIa). The displacement of a nitro group was similarly demonstrated (7).

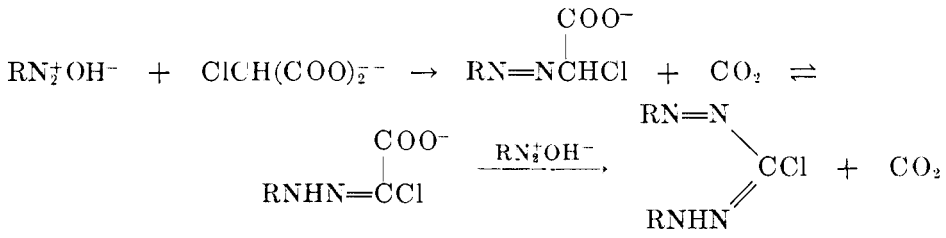


Diethyl malonate and ethyl benzoylacetate also differ from the corresponding free acids in not giving formazans by reaction with diazonium salts except under special conditions (23, 132). In most of these reactions the intermediate hydrazone can be isolated by the use of limited amounts of diazonium salt or by working at a lower pH. Ethyl cyanoacetate shows different behavior, since it yields a cyanoformazan (LIII, table 13) (158).

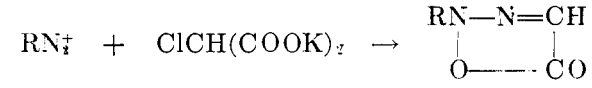


LIII

Formazans could not be obtained from ethyl chloroacetate and ethyl bromoacetate (182). The *C*-halogen formazans (table 12) were unknown until 1946, when 3-chloro-1,5-diphenylformazan was obtained by the condensation of benzenediazonium chloride with potassium chloromalonate[5] in sodium acetate solution at 0°C. (74). The reaction is said to take the following course:



A secondary reaction also takes place, leading to the formation of oxadiazolones.



In certain instances the intermediate hydrazone was isolated, and mixed chloroformazans, with two different aryl groups attached, can be prepared by the use of a second diazonium salt (75). The reaction failed to give formazans when diazotized *p*-anisidine and sulfanilic acid were used or when mono- or diesters of chloromalonic acid were used.

Phenacylmalonic acid gave the expected *C*-phenacylformazans, but failure was experienced with formylmethyl- and acetonylmalonic acids (75).

Various *C*-alkylformazans have been prepared using alkylmalonic acids. More detailed studies with allylmalonic acid (177) showed unexplained inconsistencies: diazotization and coupling with *p*-toluidine gave the expected product, 3-allyl-1,5-di-*p*-tolylformazan (table 10), but no formazan was isolated when aniline, *o*- and *m*-toluidine, α-naphthylamine, and *p*-nitroaniline were used.

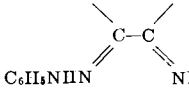
The condensation of diazonium salts with malonic acid to give formazans does not always succeed. The results obtained by Busch and Wolbring (48) showed that three possible products could be isolated from such a reaction: (*1*) formazans, (*2*) glyoxylic acid phenylhydrazones, and (*3*) azo-oximes of the type RN=NCH=NOH. Various diazonium salts give these products, as shown in table 3.

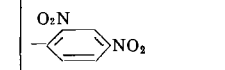
In a similar context, Whiteley and Yapp (195) also discussed the effect on

[5] In similar circumstances, potassium bromomalonate gave only triarylformazans.

TABLE 2

Preparation of formazans from compounds containing active methylene groups

Methylene Compound	Amine Diazotized	Reaction Medium	Formazan Formed		Yield	References
			N-Substituent	*C*-Substituent		
					per cent	
CH_3COCH_3	$C_6H_5NH_2$	20% NaOH	C_6H_5	CH_3CO—		(29)
	$C_6H_5NH_2$	Alkaline	C_6H_5	$C_6H_5N{=}N$—		(25)
CH_3CHO	$C_6H_5NH_2$	10% KOH	C_6H_5	$C_6H_5N{=}N$—	20-30	(18, 19, 25)
CH_3COCHO	$C_6H_5NH_2$	Alkaline	C_6H_5	$C_6H_5N{=}N$—		(25)
$CH_3COCOOH$	$C_6H_5NH_2$	10% KOH	C_6H_5	—COCOOH	94	(18, 19)
$CNCH_2COOH$	$C_6H_5NH_2$	Alkaline or pH 6	C_6H_5	—CN	97*	(93, 193)
$CNCH_2COOC_2H_5$	$C_6H_5NH_2$	Alkaline	C_6H_5	—CN		(158)
$CNCH_2CHO$	$4\text{-}BrC_6H_4NH_2$	—	$4\text{-}BrC_6H_4$	—CN		(36)
CH_3COCH_2COOH	$ArNH_2$†	Na_2CO_3	Ar	$C_6H_5N{=}N$— and CH_3CO—		(5, 6, 17, 25, 26, 93)
$CH_3COCH_2COOC_2H_5$	$C_6H_5NH_2$	10% KOH; potassium salt in CH_3COONa	C_6H_5	—$COOC_2H_5$	40-80	(6, 17, 26, 132)
$C_6H_5COCH_2COOH$	$C_6H_5NH_2$	10% KOH	C_6H_5	—COC_6H_5	41	(6, 27, 28)
$C_6H_5COCH_2COOC_2H_5$	$3\text{-}O_2NC_6H_4NH_2$	Potassium salt	$3\text{-}O_2NC_6H_4$	—COC_6H_5	Small	(23)
$CH_2(COOH)_2$	$ArNH_2$	CH_3COONa or CH_3ONa	Ar	H and $C_6H_5N{=}N$—	46 (aniline)	(48, 93, 132)
$C_2H_5OOCCH_2COOH$	$4\text{-}O_2NC_6H_4NH_2$	pH 8–9	$4\text{-}O_2NC_6H_4$	—$COOC_2H_5$	52*	(93)
$CH_2(COOC_2H_5)_2$	$C_6H_5NH_2$	Sodium salt of ester	C_6H_5	—$COOC_2H_5$	50	(132)
$RCH(COOH)_2$	$ArNH_2$	CH_3COONa	Ar	—R	50 (R = benzyl) 100 (R = ethyl)	(177)
$CH_2(CONHCOOC_2H_5)_2$	$ArNH_2$	Na_2CO_3	C_6H_5	—$CONHCOOC_2H_5$		(195)
$HOOCCH_2COCH_2COOH$	$C_6H_5NH_2$	CH_3COONa	C_6H_5	—COOH		(137)
$CH_3COCH_2CH_2COOH$	$C_6H_5NH_2$	10% KOH	$C_6H_5N{=}N$  $N{=}NC_6H_5$		10	(19)
			C_6H_5NHN	$NNHC_6H_5$		
$CH_3COCH(CH_3)COOH$	$C_6H_5NH_2$		C_6H_5	—CH_3		(18)
$CH_3COCH(CH_3)COOC_2H_5$	$C_6H_5NH_2$	Dilute KOH	Mixed products			(19)
$C_2H_5OOCCOCH_2COOC_2H_5$	$C_6H_5NH_2$	NaOH	C_6H_5	—$COOC_2H_5$	76	(146, 198)
	$4\text{-}CH_3C_6H_4NH_2$	NaOH	$4\text{-}CH_3C_6H_4$	—$COOC_2H_5$	81	(146)
	$4\text{-}BrC_6H_4NH_2$	NH_4OH	$4\text{-}BrC_6H_4$	—$COOC_2H_5$	41	(144)
$HOOCCH_2CH{=}CHCOOH$	$C_6H_5NH_2$		C_6H_5	—$CH{=}CHCOOH$		(87)
$C_2H_5OOCCH_2CH{=}CHCOOC_2H_5$	$C_6H_5NH_2$					(87)

$HOOCCH_2CH{=}C(CH_3)COOH$	$C_6H_5NH_2$	CH_3COONa	C_6H_6	$-CH{=}C(CH_3)COOH$	Good	(86)
$NaOCH{=}CHCOOC_2H_5$	$C_6H_5NH_2$	H_2O	C_6H_5	$-COOC_2H_5$		(122)
$ClCH(COOH)_2$	$C_6H_5NH_2$	CH_3COONa	C_6H_5	$-Cl$		(74, 75)
$BrCH(COOH)_2$	$C_6H_5NH_2$	CH_3COONa	—	—		(74)
$C_6H_5COCH_2CH(COOH)_2$	$C_6H_5NH_2$	$CH_3COONa+Na_2CO_3$	C_6H_5	$-CH_2COC_6H_5$		(75)
	$C_6H_5NH_2$		C_6H_5			(131)
$C_6H_5COCH_2COCH_3$	$C_6H_5NH_2$	Aqueous KOH	C_6H_5	$-COC_6H_5$	24, 38	(27, 28)
$C_6H_5N{=}NCH(COCH_3)_2$	$C_6H_5NH_2$	NaOH	C_6H_5	$-COCH_3$		(57)
$C_2H_5OOCCOCH_2CH{=}CHCOOC_2H_5$	$C_6H_5NH_2$	NH_4OH	C_6H_5	$-CH{=}CHCOOC_2H_5$	17	(144)
	4-$BrC_6H_4NH_2$		4-BrC_6H_4	$-CH{=}CHCOOC_2H_5$	8	(144)
$C_2H_5OOCCOCH_2CH{=}CHCH{=}CH-COOC_2H_5$	$C_6H_5NH_2$		C_6H_5	$-CH{=}CHCH{=}CHCOOC_2H_5$		(37)
CH_3NO_2 (see also table 12)	$C_6H_5NH_2$	$CH_3COONa + CH_3COOH$	C_6H_5	$-NO_2$	36	(7, 20, 22, 24, 72)
$NO_2CH_2CHOHCOC_6H_5$	4-$O_2NC_6H_4NH_2$	NaOH	4-$O_2NC_6H_4$	$-NO_2$		(49)
$NO_2CH_2CONH_2$	4-$O_2NC_6H_4NH_2$	pH 4	4-$O_2NC_6H_4$	$-NO_2$	Small	(93)
NO_2CH_2COOH	4-$O_2NC_6H_4NH_2$	pH 4	4-$O_2NC_6H_4$	$-NO_2$	Small	(93)
$CNCH_2CONH_2$	4-$O_2NC_6H_4NH_2$	pH 6	4-$O_2NC_6H_4$	$-CN$	Small	(93)
$ROOCCH_2CONH_2$	4-$O_2NC_6H_4NH_2$	pH 7	4-$O_2NC_6H_4$	$-COOR$	Small	(93)
NH_2COCH_2COOH	4-$O_2NC_6H_4NH_2$	pH 6	4-$O_2NC_6H_4$	$-CONH_2$	89*	(93)
$CNCH_2SOCH_3$	4-$O_2NC_6H_4NH_2$	—	4-$O_2NC_6H_4$	$-CN$	Good	(93)
$NH_2COCH_2SO_2CH_3$	4-$O_2NC_6H_4NH_2$	pH 8	4-$O_2NC_6H_4$	$-SO_2CH_3$	54	(93)
$CH_3SO_2CH_2SOCH_3$	4-$O_2NC_6H_4NH_2$	—	4-$O_2NC_6H_4$	$-SO_2CH_3$	Small	(93)
$NH_2COCH_2CONH_2$	4-$O_2NC_6H_4NH_2$	pH 8	4-$O_2NC_6H_4$	$-CONH_2$	Small	(93)

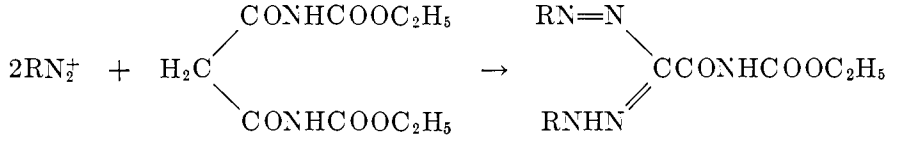
* Crude yield. † Ar = aryl.

TABLE 3

Products of the condensation of malonic acid with diazonium salts

Amine Diazotized	Products		
	Formazans	Glyoxylic acid phenylhydrazones	Azo-oximes
o-Nitroaniline	5 per cent	50 per cent	
m-Nitroaniline	Sole product; small yield		
p-Nitroaniline	Major product	Small	
o-Chloroaniline			Sole product
o-Bromoaniline		Sole product (30–40%)	
p-Bromoaniline	Sole product		
o-Iodoaniline	Major product	Small	
o-Toluidine			Sole product
m-Toluidine			
p-Toluidine	Major product		
o-Anisidine			Sole product
p-Anisidine	Major product		
α-Naphthylamine			Sole product
β-Naphthylamine			Sole product
2,4-Dimethylaniline			Sole product

formazan formation of substitution in the diazonium salt. From studies of condensations with malonyldiurethan, they concluded that formazan formation occurs when a nucleophilic group is present in the para position or when an electrophilic group is in the ortho position in the diazonium salt. It is hindered, however, by an electrophilic group in the meta or para position. They further showed that formazans (table 13) resulted when the reaction was carried out in sodium carbonate solution, but hydrazones were obtained when acetic acid was used as solvent.



Recently, Hünig and Boes (93) have published a detailed study of the relative ability of different methylene groups to couple with diazonium salts. This reaction was chosen since it possessed characteristics suitable for the assessment of the relative reactivity of methylene compounds, XCH_2Y, where X and Y were both varied. Studies were made where X and Y were variously NO_2, CHO, $COCH_3$, CN, COOR, $CONH_2$, COOH, SO_2CH_3, COC_6H_5, $SO_2C_7H_7$, $SO_2C_6H_5$, $SOCH_3$, and C_6H_5. Most of the possible methylene compounds in a list of fifty-five were studied using *p*-nitrobenzenediazonium fluoborate, because of its stability and its ability to couple readily and give well-crystallized products. The coupling was investigated over a pH range of 2–10. The lowest pH at which a compound coupled was indicative of its reactivity; only very reactive methylene compounds coupled in acid solution. The order of substituents given above is roughly the order of their decreasing ability to activate. Although hydrazones of the type

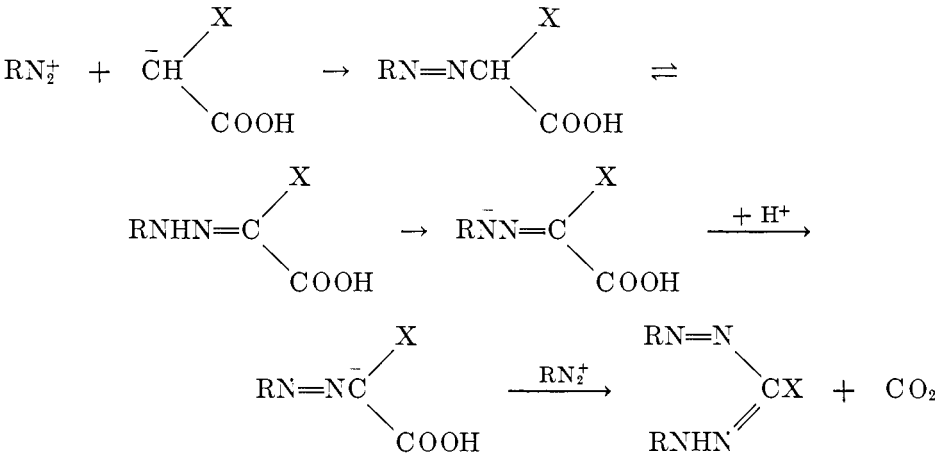
RNHN=CXY are the primary products, formazans are formed as secondary products in those cases where either X or Y can be displaced by a diazonium ion. This displacement depends on the pH, but can occur for the following pairs:

X	Y	X	Y	X	Y
NO_2	$CONH_2$	COOR	$CONH_2$	COOH	COOH*
NO_2	COOH	CN	COOH*	$CONH_2$	SO_2CH_3*
$COCH_3$	COOH	COOR	COOH*	CH_3SO_2	$SOCH_3$
CN	$CONH_2$	$CONH_2$	$CONH_2$	CN	$SOCH_3$*
		$CONH_2$	COOH*		

* In the examples marked with an asterisk, the formazan is the major product.

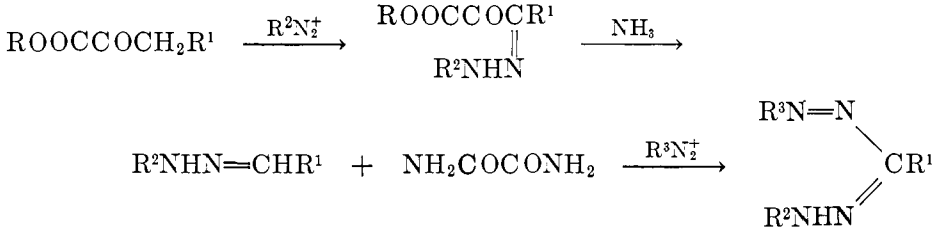
In a further discussion of the mechanism of the reaction the authors suggested that four factors influence the observed "activity" of methylene compounds: (*1*) the acidity of the CH form, (*2*) the rate of dissociation of the CH form, (*3*) the amount of enol form present, and (*4*) the acidity of the enol form.

Formazan formation appears to result by the following reaction mechanism:



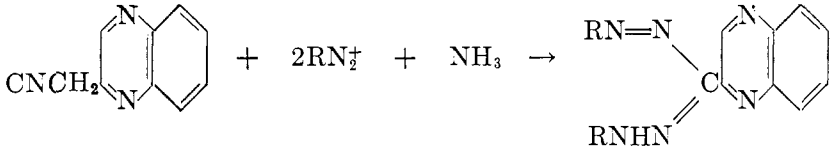
The condensation at the second step involves the spontaneously dissociated, weakly acidic NH group, and explains why formazan formation is so dependent upon the pH. This view is different from that postulated by Busch for aldehyde phenylhydrazones (page 380), which involves the formation of an unstable tetrazene as intermediate; yet the latter mechanism was accepted by Hünig and Boes as explaining the formation of a tetrazolium betaine as a by-product in the coupling of *p*-nitrobenzenediazonium salts with a β-disulfone (page 408).

A useful variation of this general method of formazan synthesis has recently been reported (156). When *C*-heterocyclic formazans (table 19) are required, the inaccessible heterocyclic aldehydes may often be avoided by the condensation of diazonium salts with heterocyclic pyruvic esters in ammonia solution.



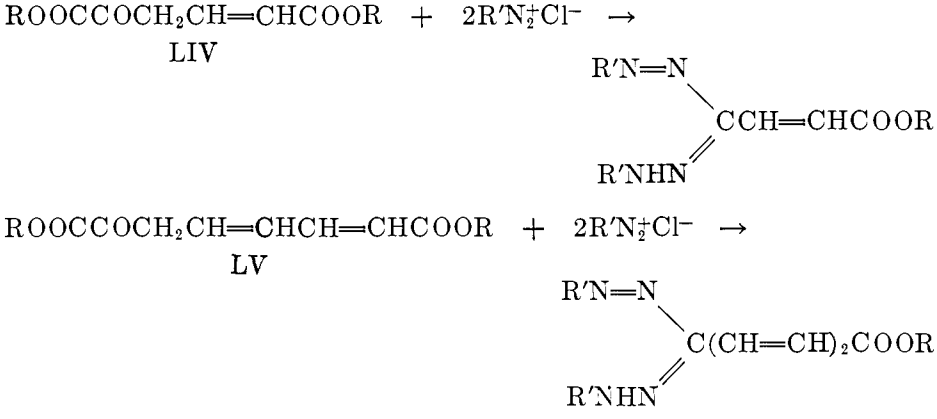
The pyruvic esters are readily obtained from heterocyclic compounds containing active methylene groups, by condensing the latter with diethyl oxalate.

Similarly, heterocyclic acetonitriles have been converted to formazans in a few cases.

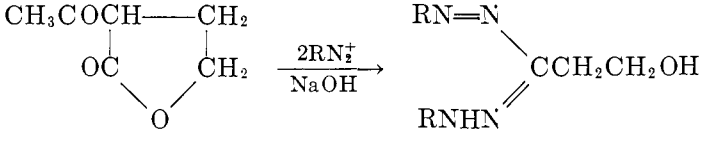


Other cases failed, the intermediate cyanohydrazone being isolated.

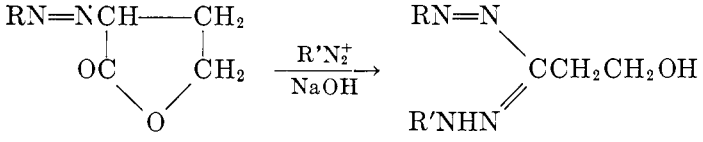
The preparation of formazans from oxalocrotonic ester (LIV) (144) and from oxalosorbic ester (LV) (table 13) (37) follows a similar course, with the loss of oxamide.



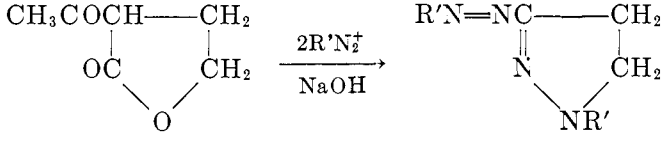
Duffin and Kendall (60a) have recently studied a formazan synthesis related to the condensation of the diazonium salt with the active methylene compound, although the attack is actually on a methine carbon atom. α-Aceto-γ-butyrolactone was condensed with diazonium salts carrying ortho substituents in the aromatic ring, in strongly alkaline solution, to give good yields of 1,5-diaryl-3-β-hydroxyethylformazans.



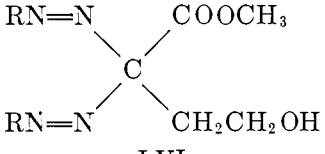
where R = *o*-tolyl, *o*-chloro-, *o*-methoxy-, and *o*-nitrophenyl. α-Aceto-γ-valerolactone similarly leads to 3-β-hydroxypropylformazans. The aryl substituents in formazans of this type have been varied by the condensation of ortho-substituted diazonium salts with arylazobutyrolactones or -valerolactones.



If the diazonium salts used are not ortho-substituted, the products of condensation are 3-arylazopyrazolines.

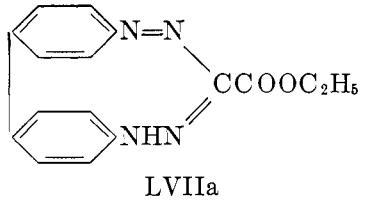


where R′ = phenyl, *m*-tolyl, *p*-tolyl, *m*-chlorophenyl, *p*-chlorophenyl, *p*-bromophenyl, and *p*-methoxyphenyl. In the presence of limited amounts of alkali, the lactones react with ortho-substituted diazonium salts to give a crude product which is converted by treatment with methanol to a red compound, which appears to be LVI and which is converted to a pyrazoline by sodium hydroxide in ethanol.

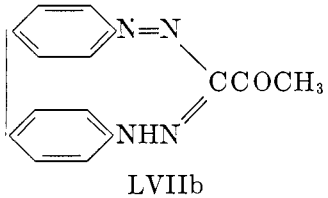


LVI

The condensation of tetrazotized benzidine with ethyl acetoacetate in dilute potassium hydroxide solution was studied by Wedekind (190). The product was said to be ethyl cycloformazylformate (LVIIa), but a subsequent paper (186) claimed that cycloformazyl methyl ketone (LVIIb) was produced.



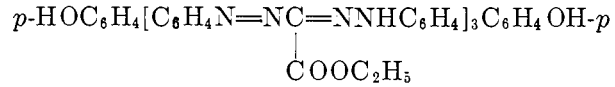
LVIIa



LVIIb

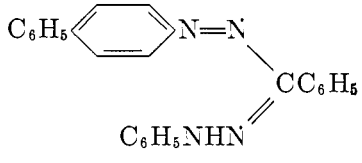
These structures seem unlikely on stereochemical grounds. They were unsubstantiated, the analyses were poor, and the nature of the tetrazolium salt obtained by oxidation was not made clear. Recent reinvestigation of the problem (200) has shown that the product is non-homogeneous and has a molecular

weight (by the Rast method in camphor) of about 1000. The main constituent was shown, after a chromatographic separation on alumina, to be LVIII.

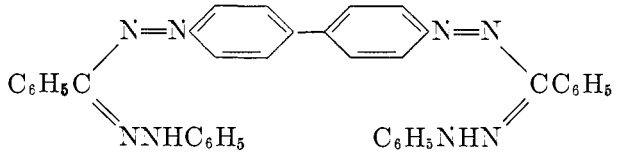


LVIII

Wedekind also claimed that tetrazotized benzidine could be condensed with benzaldehyde phenylhydrazone to give LIX (184, 186), one of the diazonium groups having suffered reduction.



LIX



LX

The properties of his product, however, were not the same as those of the compound recently obtained from the condensation of diazotized 4-aminobiphenyl and the same phenylhydrazone (2, 100). The formazan LX (table 21), which might be expected from Wedekind's reaction, which was said to have given LVII, was actually prepared from tetrazotized benzidine and phenylglyoxylic acid phenylhydrazone (186).

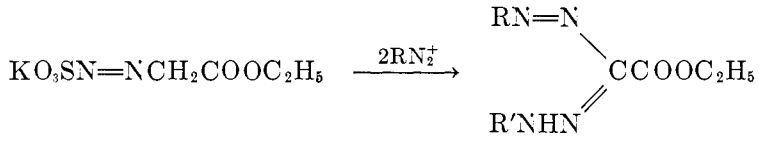
Formazans have also been prepared by the action of diazonium salts on various other compounds which do not fall into the above groups.

(b) By the action of diazonium salts with aliphatic azo compounds

Diazonium salts can be condensed with aliphatic azo compounds which contain replaceable groups. Most of these compounds are intermediates in many of the preparations described in method 2, but some have been isolated and reacted separately with a further molecule of a (different) diazonium salt (15). Two different aryl groups can thereby be introduced into aliphatic molecules. Benzeneazoacetoacetic ester reacts with diazonium salts in this way.

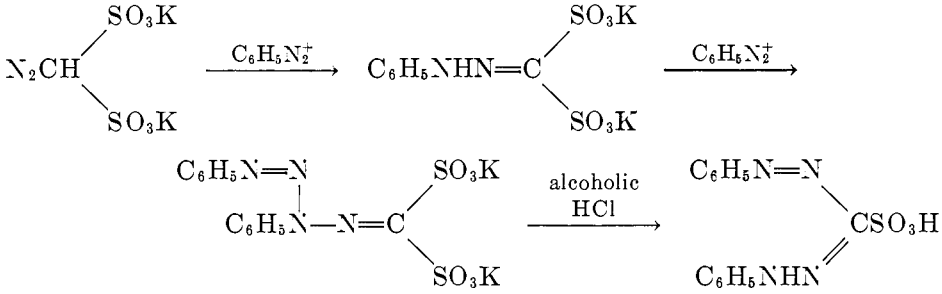
(c) By the action of diazonium salts with the sulfonic acid derivative of ethyl diazoacetate

The sulfonic acid derivative of ethyl diazoacetate (LXI) reacts with benzenediazonium chloride in alkaline solution (136).



LXI

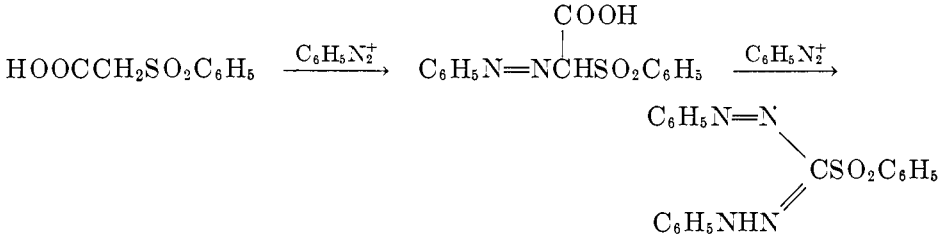
A formazan of a type not otherwise described (LXII; table 12) is obtained when diazomethanedisulfonic acid is reacted with a diazonium salt.



LXII

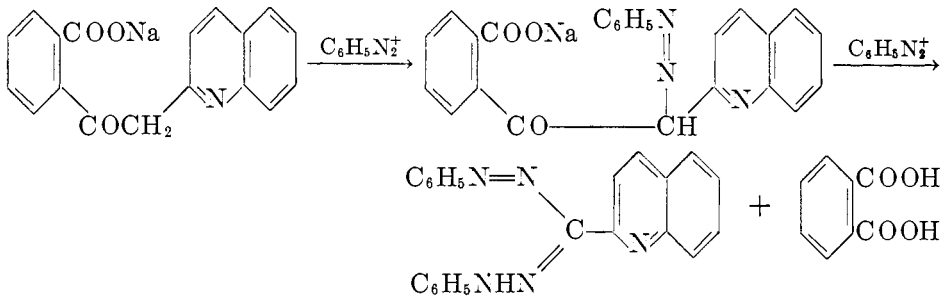
(d) By the action of diazonium salts with benzenesulfonylacetic acid

Benzenesulfonylacetic acid has been shown to react with diazonium salts in the way that acetoacetic acid does, but some failures were noticed (175). Formazans (table 13) were isolated using *o*-anisidine and *o*-toluidine, but no products were isolated in the cases of aniline, *m*- and *p*-toluidine, *p*-anisidine, and *p*-phenetidine; nor were formazans produced when *p*-toluenesulfonylacetic acid was used.



(e) By the action of diazonium salts with isoquinophthalones

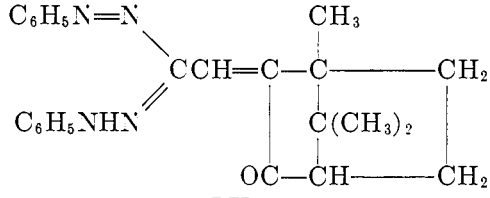
Isoquinophthalones and diazonium salts give *C*-quinolylformazans (table 19) by a two-stage reaction (61).



This is a special variant of the active methylene type of condensation.

(f) By the action of diazonium salts with β-camphorylidenepropionic acid

A terpene nucleus has been introduced into the formazan molecule (159) (table 26) by the action of benzenediazonium chloride on β-camphorylidenepropionic acid (LXIII).



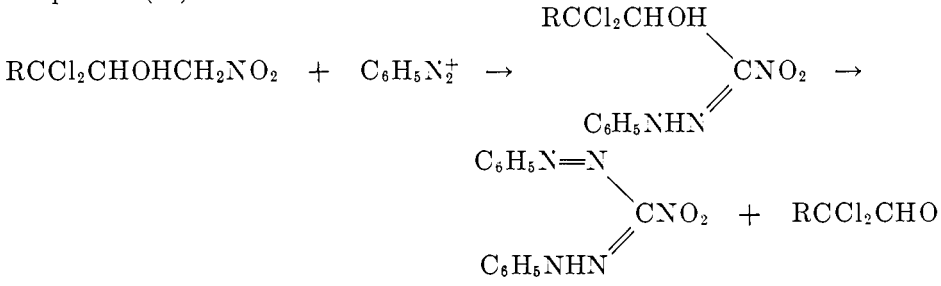
LXIII

(g) By the action of diazonium salts with unsaturated phenols

Diazonium salts react with unsaturated phenols of the safrole type to yield 1,5-diaryl-3-methylformazans (145).

(h) By the action of diazonium salts with trichloro-β-hydroxy-α-nitroparaffins

Diazonium salts react with compounds of the trichloro-β-hydroxy-α-nitroparaffin type to give formazans, but not with acetates or other esters of these compounds (54).

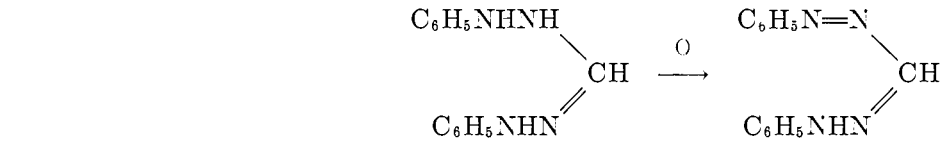


B. FROM ARYLHYDRAZINES (METHOD B)

Phenylhydrazine reacts with a variety of compounds to form formazans. Two molecules of phenylhydrazine are required, one forming a hydrazone group and the other a hydrazo group; the latter becomes oxidized to an azo group in the course of the reaction. On occasion, the azo group is already present in the reacting molecule.

1. By the action of phenylhydrazine with ethyl formate and ethyl orthoformate

Ethyl formate (132, 133) and ethyl orthoformate (58, 179) react with phenylhydrazine to give 1,5-diphenylformazan (table 9).



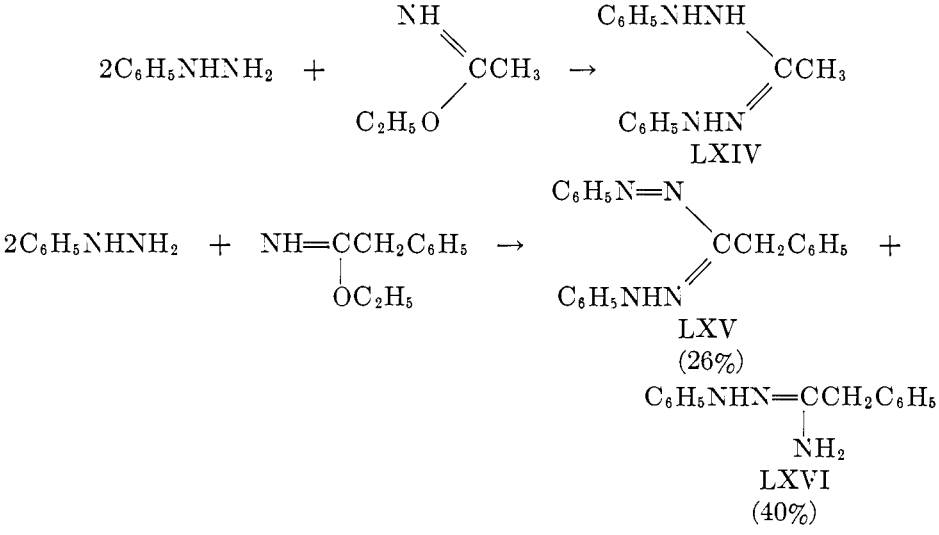
This is a special case of a more general reaction, which was referred to by von Pechmann in his list of formazan syntheses (133) but which has not otherwise been tested experimentally. In this hypothetical synthesis it was implied that phenylhydrazine would react with acylphenylhydrazides in general; no formazan was formed, however, when phenylhydrazine and β-acetylphenylhydrazine were heated together in ethanol (126a).

2. By the action of phenylhydrazine with ethyl nitrate

Ethyl nitrate and phenylhydrazine give 3-methyl-1,5-diphenylformazan (10, 11; table 10). Condensation with the ethyl radical appears to take place.

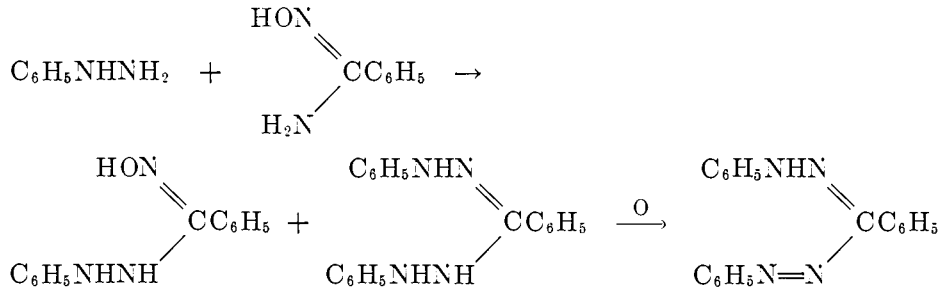
3. By the action of phenylhydrazines with imino ethers

Imino ethers and phenylhydrazines react to give formazans (101, 142, 176). The method suffers from the disadvantage that amidrazones are usually produced at the same time; indeed, the reaction has been used to obtain these compounds (101). The products vary according to the imino ether used; acetimino ethyl ether gives the hydrazidine (LXIV), which can be readily oxidized (hydrazo-azo oxidation) to the corresponding formazan. Phenacetimino ether hydrochloride and phenylhydrazine give a mixture of the *C*-benzylformazan (LXV) (table 10) and the related amidrazone (LXVI) (101, 176).



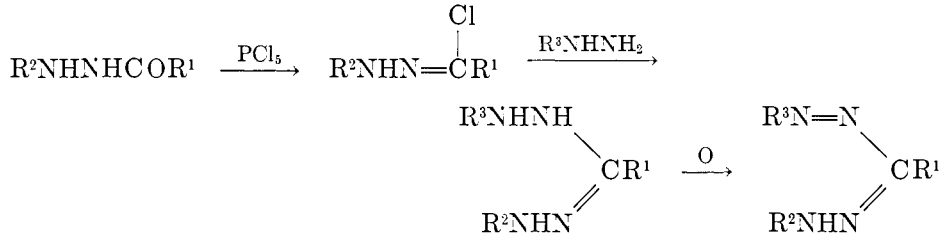
Similar mixed products have been isolated with *p*-chlorobenzimino ether and *p*-methoxybenzimino ether (101). Benzimino ether itself appears to give only the amidrazone with phenylhydrazine and some other hydrazines (but see 142), but is reported to give a mixture of formazan (not described) and amidrazone on standing for 8 days with 2,4-dichlorophenylhydrazine (101).

The reaction of hydroxyiminobenzylamine with phenylhydrazine is somewhat similar (7).



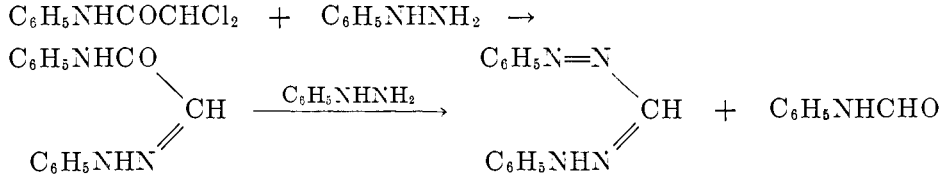
4. By the action of phenylhydrazines with halogenohydrazides

The reaction of halogenohydrazides with phenylhydrazines (55, 133) yields formazans; this reaction, potentially of wide application, has only been reported in two cases, where $R^1 = R^2 = R^3$ = phenyl and where $R^1 = R^2$ = phenyl and R^3 = 2,4-dibromophenyl.

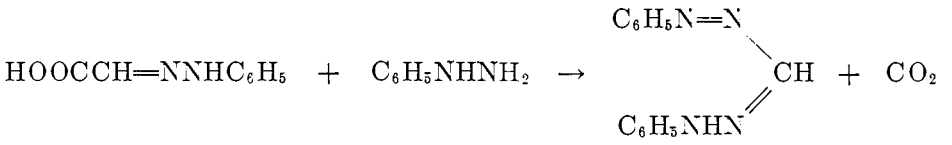


5. By the action of phenylhydrazine with dichloroacetanilide

1,5-Diphenylformazan results from heating dichloroacetanilide with phenylhydrazine (39). The reaction appears to follow the course:

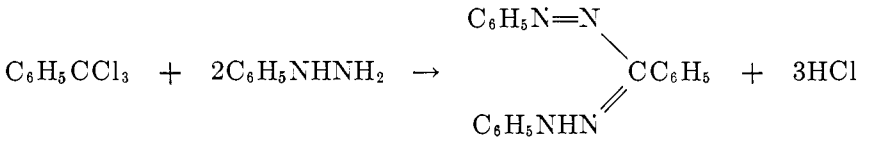


This view of the mechanism is supported by the observation of the same workers that glyoxylic acid phenylhydrazone also reacts with phenylhydrazine to give the same formazan.



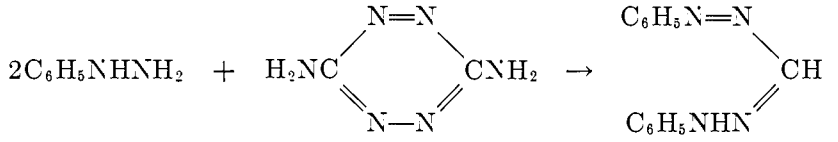
6. By the action of phenylhydrazine with benzotrichloride

Phenylhydrazine and benzotrichloride have been reported to give a good yield of triphenylformazan when heated together in alcoholic solution (36).



7. By the action of phenylhydrazine with diaminotetrazine

sym-Diaminotetrazine reacts with phenylhydrazine in 50 per cent acetic acid solution to give 1,5-diphenylformazan; *p*-bromophenylhydrazine behaves analogously.



The mechanism of the reaction has been discussed in detail (143). Recently (117a) doubt has been cast on the authenticity of the *sym*-diaminotetrazine used, which was prepared by the action of potassium hydroxide on aminoguanidine hydrochloride.

C. BY THE REDUCTION OF TETRAZOLIUM SALTS (METHOD C)

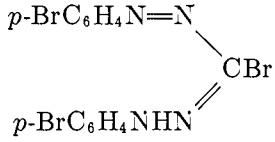
Formazans can be prepared by the reduction of tetrazolium salts. Since there is no synthesis of tetrazolium salts other than through formazans, this method is generally of no value. In certain cases, where substituents in aromatic rings have to be protected during formazan synthesis to avoid side reactions, it may be that the removal of such protection requires conditions too drastic to be applied to a molecule as reactive as a formazan. In such cases, the protected formazan can be oxidized to the corresponding tetrazolium salt, the protection removed, and the salt reduced back to the formazan. This procedure is necessary in the case of amino groups and may be convenient in certain instances with hydroxyl groups. Thus, *p*-phenetidine or *o*-benzyloxyaniline can be diazotized and coupled to give formazans in cases where the corresponding aminophenols fail. The ether groups can be removed at the tetrazolium stage by fission with halogen acids (see page 423) but not at the formazan stage (126, 141, 183).

Agents for the reduction of tetrazolium salts must be chosen with care because the formazan can be further reduced without difficulty, especially if it is some-

what soluble in the reduction medium. Although a wide variety of substances will cause reduction at pH values above 7, ammonium polysulfide, sodium amalgam, and, more recently, ascorbic acid in dilute sodium hydroxide solution (100) have been used to prepare formazans. Alkaline solutions of hydroxylamine and hydrazine are also effective. Sodium dithionite is an efficient reducing agent which can be used when the formazan is insoluble in water, but it further reduces soluble formazans to colorless compounds.

D. BY THE MODIFICATION OF SUBSTITUENTS ALREADY PRESENT IN FORMAZANS (METHOD D)

Formazans may be converted to other formazans by effecting changes in functional groups substituted in the molecule, with the limitations indicated in method C. The hydrolysis of ester and nitrile substituents to the carboxylic acids (132) and of *N*-acyl groups to free amino groups have been reported in particular cases. Nitro groups have been reduced to amines (20), carboxylic acids have been esterified through the silver salts (132), and the decarboxylation of *C*-carboxyl compounds (26, 132), has been described. In *C*-nitroformazans (table 12) the nitro group can be replaced by NH_2, SH, or OH (20); in *C*-chloroformazans the chlorine atom is reactive and has been replaced by I, NH_2, OH, and SH (74). The bromination of 1,5-diphenylformazan not only replaces the hydrogen attached to the formazan carbon by bromine, but also leads to substitution of the two benzene rings in the para positions, giving LXVII, which is identical with the product obtained by brominating 1,5-di-*p*-bromophenylformazan (96) (table 12).



LXVII

The *C*-bromo atom was as reactive as the chlorine atom in the corresponding chloro compounds, and was replaced by sodium hydrosulfide in ethanol to yield the mercaptoformazan. Formazans derived from secondary hydrazones, which cannot be prepared by the direct method, have been made by the methylation of sodium salts (147). Sometimes this method leads to inseparable mixtures.

E. BY THE OXIDATION OF DIPHENYLCARBAZIDES AND DIPHENYLTHIOCARBAZIDES (METHOD E)

Diphenylcarbazide and diphenylthiocarbazide and their ring-substituted derivatives can be oxidized at an alkaline pH to hydroxy- and mercaptoformazans (9, 20, 51, 52, 53, 63, 68, 69, 71, 85, 105, 129) (table 12).

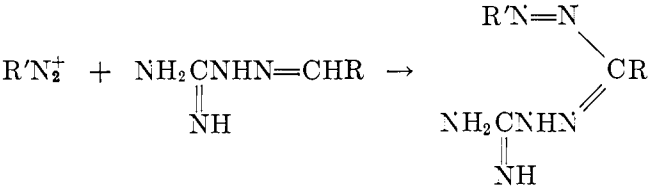
$$C_6H_5NHNHCONHNHC_6H_5 \xrightarrow[O]{KOH} C_6H_5N{=}NC(OH){=}NNHC_6H_5$$

Perhydrol is an efficient oxidizing agent (20).

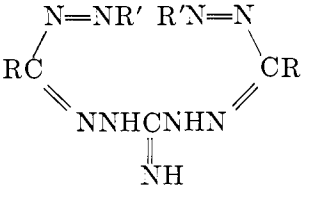
The intermediate carbazides are most easily obtained by the condensation of phenylhydrazine with phosgene (85), thiophosgene, or carbon disulfide (62). The chemistry of these compounds was investigated extensively by Bamberger, Padova, and Ormerod (20). Irving and Bell (96) have recently compared the routes to the mercaptoformazans and concluded that the one involving the action of hydrosulfides on *C*-chloroformazans was the best. They discussed the chemistry of the "dithizones" or mercaptoformazans in detail.

F. THE SYNTHESIS OF GUANAZYLS (METHOD F)

Guanazyls (table 22) are obtained by a modification of method A in which aldehyde or pyruvic acid guanylhydrazones react with diazonium salts (181, 185, 189).



This reaction failed when R was CH_3 and R′ was phenyl or *p*-tolyl, but succeeded when R was CH_3 and R′ was *m*-nitrophenyl. There are probably fewer limitations when R and R′ are both aryl groups. Scott, O'Sullivan, and Reilly (161, 162, 163) prepared a large number of bisguanazyls of the type of LXVIII from diaminoguanidine, including a number of heterocyclic derivatives.



LXVIII

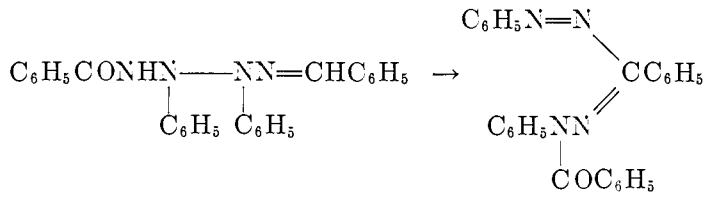
These authors discussed the reaction mechanism in some detail; in considering the condensation of the diazonium salt with aldehyde phenylhydrazone or of diazonium salt with benzalaminoguanidine, they drew attention to three possible reactions occurring in rivalry: (*i*) coupling with the aromatic rings; (*ii*) formazan formation, involving coupling with the CH group; (*iii*) tetrazene formation involving coupling with the NH group. This seems to ignore the views of Busch (43, 46), since it regards (*ii*) and (*iii*) as rival processes, whereas Busch considered (*iii*) to be an essential step to (*ii*) and supported his view with impressive experimental evidence (page 380). Scott, O'Sullivan, and Reilly further argue that electronegative substituents in the aldehyde moiety should enhance formazan formation, which should also be enhanced by the use, in the hydrazine moiety, of derivatives of hydrazinocarbonic acid. Although they demonstrated the successful synthesis of formazans from the aminoguanyl derivatives of alde-

hydes (a special case of a carbonic acid derivative), they admit that, contrary to their hypothesis, aldehyde acylhydrazones do not yield formazans at all.

G. MISCELLANEOUS FORMAZAN SYNTHESES (METHOD G)

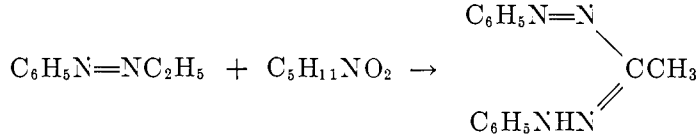
Formazans have been produced by the following miscellaneous reactions:

(*1*) The action of heat on "tetrazan" (42):



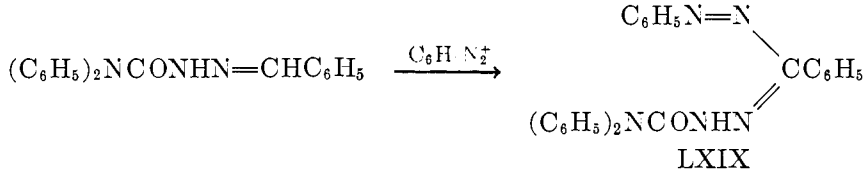
This reaction gives rise to *N*-acylated formazans.

(*2*) The condensation of phenylazoethane with isoamyl nitrite in the presence of hydrogen chloride leads to 3-methyl-1,5-diphenylformazan (21).



Phenylazoethane also couples with benzenediazonium chloride to yield 1,5-diphenyl-3-phenylazoformazan (table 18).

(*3*) Aldehyde semicarbazones in which the amino group (as distinct from the hydrazino group) is substituted with two aryl radicals couple with diazonium salts to give formazan-like compounds of the type of LXIX (table 25) (155).



LXIX

Thiosemicarbazones also appear to react in this way, although no compounds derived from them have been described. In either case, the terminal amino group must be disubstituted with aryl groups for formazan formation to take place. No formazans were obtained from compounds of the following types:

LXXa: $RCH{=}NNHCOC_6H_5$

LXXb: $RCH{=}NNHCSC_6H_5$

LXXc: $RCH{=}NNHCSSC_2H_5$

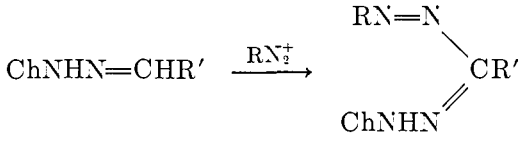
LXXd: $RCH{=}NNHCSNHN{=}CHR$

LXXe: $RCH{=}NNHCSOC_2H_5$

Although these compounds have a formal resemblance to arylidenephenylhy-

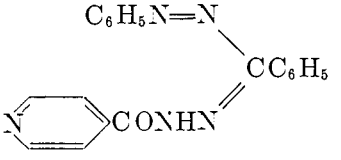
drazines, the inability to undergo formazan formation may be due to the imido group being principally in the enol form.

Although acylhydrazides (LXXa) fail to react with diazonium salts to give formazans, the formation of "cholylformazans" by the action of diazonium salts on cholylhydrazones (a special case of LXXa, reported by Capka (50)) appears to be an exception. The chemical identity of the cholylformazans is, however, not well substantiated.



Ch = cholyl.

An attempt to condense benzenediazonium chloride with benzaldehyde isonicotinylhydrazone failed to produce any of the expected formazan (LXXI) (126a).



LXXI

Such a result is to be anticipated if Busch's view of formazan formation (page 380) is correct and the imido nitrogen is deprived of all basic character.

VII. Synthesis of Tetrazolium Salts

All the tetrazolium salts which have been reported have been prepared, directly or indirectly, by the oxidation of formazans; some of them have been obtained by the modification of structures present in an already existent tetrazolium nucleus.

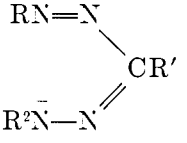
The oxidation of formazans may be performed in a variety of ways. The first reagent to be used (138, 140) was nitrous fumes, but subsequently mercuric oxide or isoamyl nitrite with an acid was used successfully. More recently, lead tetraacetate (106) was shown to give good yields of a more pure product, whilst Kuhn and Münzing (108) have described the smooth and rapid reaction of formazans with halogenoamides to give excellent yields of tetrazolium salts. The method to be applied must be determined empirically, and it is difficult to predict which will be the best reagent in any given case.

Mercuric oxide may be used in alcoholic or in chloroform solution; the oxidation is complete after heating for widely varying intervals of time and is shown by the disappearance of formazan color. The tetrazolium hydroxide is produced in solution and may be separated from a sludge of metallic mercury and mercuric oxide. The method has the advantage that various salts are readily ob-

tained by neutralization with the appropriate acid, but when heating has to be prolonged, the tetrazolium hydroxide may suffer some decomposition, with the result that poor yields or impure products are obtained. Mercuric chloride is also effective as an oxidizing agent and leads to the chloride, the reaction sometimes being more rapid than with the oxide.

Isoamyl nitrite is used in alcoholic solution, cooled to about 0°C. in the presence of anhydrous or aqueous hydrogen chloride, sulfuric acid, or glacial acetic acid. After the addition of water, the product is best worked up by the removal of water and isoamyl alcohol *in vacuo*. The active oxidizing agent appears to be nitrous acid, generated *in situ*. Ethyl and butyl nitrite have been used successfully, but it is noteworthy that the oxidation of 3-methyl-1,5-diphenylformazan proceeded normally to the tetrazolium salt with isoamyl nitrite, and yet gave an abnormal product (which was not identified) with nitrous fumes. The oxidation of the formazan (LXXIII) (page 405) with isoamyl nitrite caused concomitant nitrosation; the nitrosotetrazolium salt was hydrolyzed to the required unsubstituted product by boiling with ethanolic hydrogen chloride (118) (table 32).

Lead tetraacetate is usually the reagent of choice. It reacts fairly rapidly and smoothly in chloroform solution either at room temperature or in the boiling solvent. When the formazan is completely insoluble in chloroform (126), cold glacial acetic acid can be used as solvent. The removal of lead occasionally presents difficulties. Oxidation in a closed system has been shown to involve a marked uptake of oxygen and presumably proceeds *via* the odd-electron intermediate (LXXII) (2).



LXXII

The tetrazolium acetate is the immediate product and can be converted to salts such as the chloride by the addition of enough hydrochloric acid to precipitate the lead and to replace the acetic acid, which is then removed by evaporation *in vacuo*. In many cases, the tetrazolium acetate crystallizes satisfactorily.

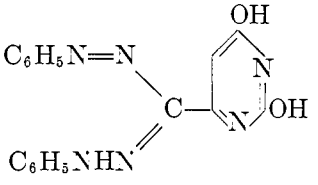
N-Bromosuccinimide, *N*-chloroacetamide, *N*-bromophthalimide, and *N*-chlorosuccinimide have been reacted in alcoholic solution with formazans (108). The tetrazolium halide is usually formed rapidly and can readily be separated from the parent amide or imide in a state of considerable purity. Triphenyltetrazolium bromide has been prepared in a pure condition in almost quantitative yield by this method.

The use of *tert*-butyl hypochlorite as an oxidizing agent for formazans has recently been reported (32a). The tetrazolium chloride is readily isolated in yields of 60 per cent when the reactants are mixed in chloroform or dioxane solution.

Other oxidizing agents have been employed, but some of them lead to in-

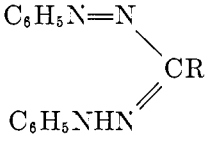
soluble tetrazolium salts which are not convenient for further work. Thus, chromium trioxide in glacial acetic acid readily converts formazans to tetrazolium dichromates, and sodium hypochlorite forms tetrazolium chlorates. Chlorine rapidly decolorizes formazan solutions but readily effects simultaneous nuclear chlorination. Hydrogen peroxide has no effect on formazans unless suitable catalysts are present, notably ferrous ions (2) or vanadium pentoxide (152).

In certain cases, aerial oxidation is effective. It has been applied as a preparative procedure to 3-hydroxy- and 3-mercapto-1,5-diphenylformazan (20, 105a) and observed to be facile with 3-methyl-1,5-diphenylformazan (18), which is unstable on keeping and undergoes slow autoxidation in air. Krumholz and Watzek (105a) studied the slow autoxidation of aqueous alkaline solutions of diphenylcarbazone (3-hydroxy-1,5-diphenylformazan) and found that the process was more rapid in ammonia solution and in the presence of cupric ions. Under these conditions, 5-hydroxy-2,3-diphenyltetrazolium betaine was prepared in 50 per cent yield. At a lower pH, a disproportionation of formazan to a mixture of diphenylcarbazide and the betaine occurs. Many other formazans show indications of slow but definite autoxidation. Ludolphy (118) prepared the pyrimidylformazan (LXXIII, table 19) by the usual condensation between a diazonium salt and an aldehyde phenylhydrazone but found that the formazan was so readily oxidized that air had to be excluded from the reaction during the preparation. Aerial oxidation was, however, not satisfactory as a preparative route to the corresponding tetrazolium salt.



LXXIII

Some comparative studies of the oxidative ring-closure of formazans have been made by Wedekind (191). He explained his observations in terms of the effect of the various groups, attached to the 3-carbon atom, on the ease of rotation of the formazan chain. The more readily the substituent R in LXXIV permits rotation, the slower is the oxidation.

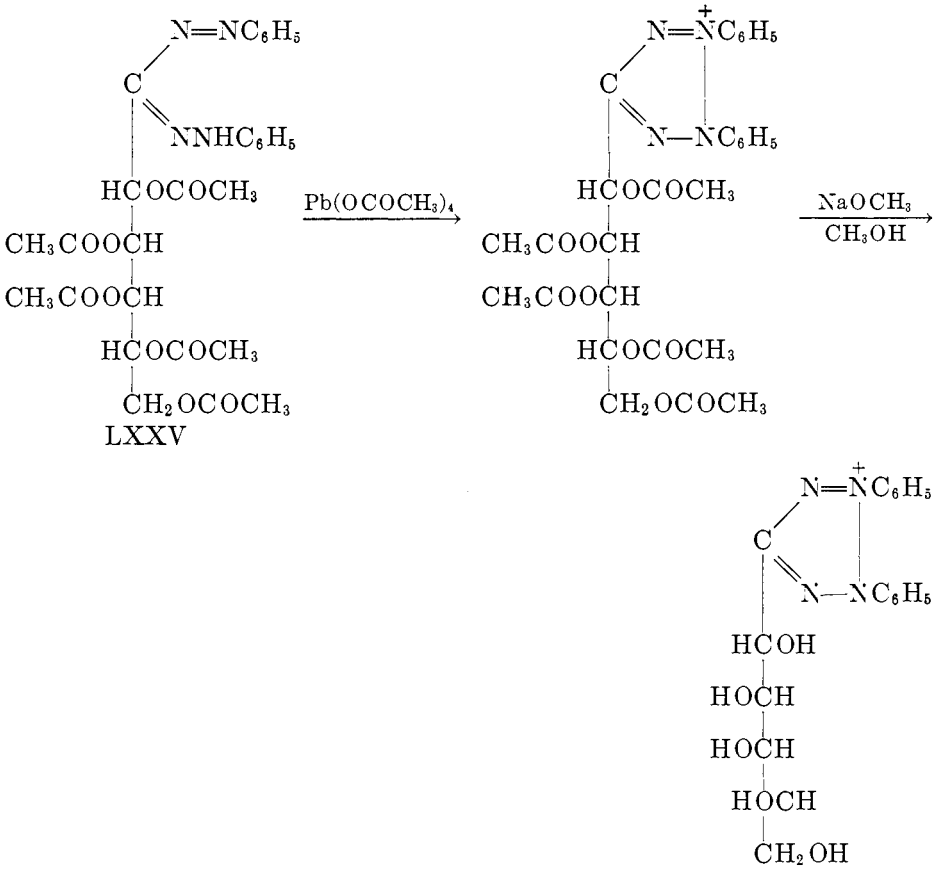


LXXIV

The order of the series R = H, COOH, CH_3, $COCH_3$, $N{=}NC_6H_5$, COC_6H_5, $COOC_2H_5$, C_6H_5, and CN corresponds to the order of increasing yield of tetrazolium salt resulting from the oxidation of LXXIV. It was not found practicable to follow these oxidations in more detail by colorimetric methods.

In studies of the effect of substituents in the *N*-phenyl groups upon the rate of oxidation, the reaction rate was reduced to a measurable quantity by studying formazans derived from *m*-carboxyphenylhydrazones. In the case of those derived from phenylhydrazine, the reaction proceeded too rapidly for measurements to be made. Variation of the substituents in the other *N*-phenyl ring (achieved by the use of different benzenediazonium salts) showed that the yield upon oxidation to a tetrazolium salt was reduced by electrophilic substituents. This effect also depended on the position of substitution and was greatest in the ortho position and least in the meta position. It was observed most markedly with nitro groups and less clearly with carboxyl groups, whilst the *o*-chloro compound had a much smaller influence. The methyl and *p*-isopropyl groups produced the same effect as a simple phenyl group.

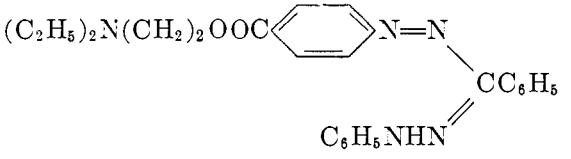
There do not appear to have been any cases reported in which a formazan could not be oxidized to a tetrazolium salt. The oxidation of formazans linked to a sugar molecule (e.g., LXXV) to the corresponding tetrazolium salts could only be achieved when the hydroxyl groups had been protected by acetylation. Oxidation by lead tetraacetate then readily gave a good yield of the penta-acetyltetrazolium salt, which could readily be hydrolyzed to the free sugar-tetrazolium salt (205; table 35):



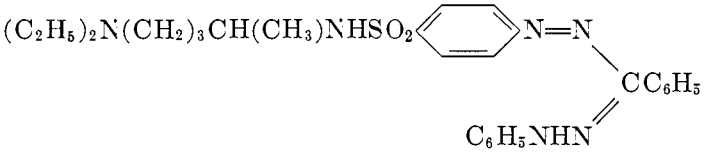
LXXV

Without such protection, the nitrogen-containing fragments of the molecule were split off, and in the case of *d*-mannodiphenylformazan, *d*-mannonic acid γ-lactone (see page 412) was formed. These sugar-tetrazolium salts can be reduced back to the formazan by ascorbic acid in the normal manner.

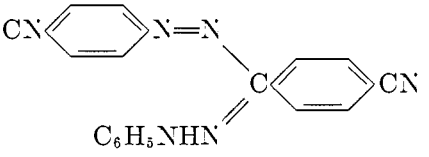
Failures to isolate the tetrazolium salt in some cases have been attributed to difficulties of purification and not of oxidation. Such failures were reported (2) for the β-diethylaminoethyl ester of 1-*p*-carboxyphenyl-3,5-diphenylformazan (LXXVI, table 16), 1,3-diphenyl-5-*p*-sulfon(5-diethylaminopent-2-yl)amidophenylformazan (LXXVII, table 16), and 1,3-di-*p*-cyanophenyl-5-phenylformazan (LXXVIII, table 16). In the first two cases, the tetrazolium salts would be expected *a priori* to crystallize with difficulty; even in the absence of groups which make the process difficult, the crystallization of tetrazolium salts is often slow and tedious.



LXXVI

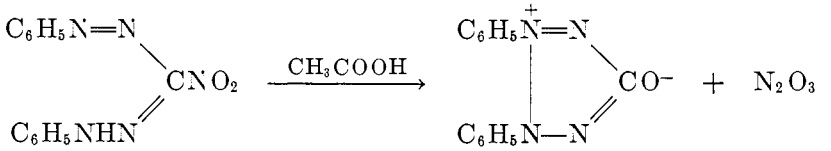


LXXVII



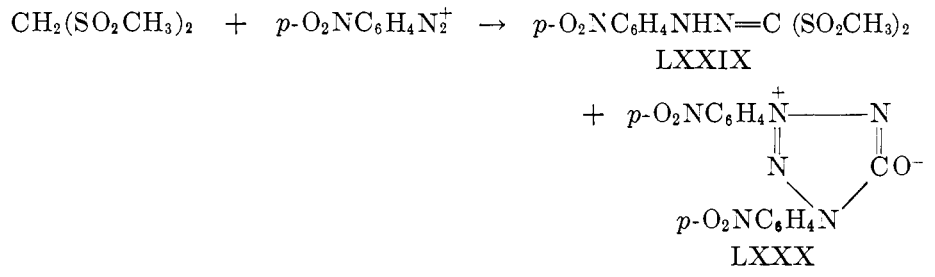
LXXVIII

New tetrazolium salts have been prepared in a number of cases by the modification of substituent groups in existent tetrazolium molecules. Examples have been described in which acetamido groups were hydrolyzed to amino groups (2), ethoxyl groups split with concentrated hydrobromic acid to hydroxyl groups (141), and cyano groups hydrolyzed with hydrochloric acid to carboxyl groups (126). Free amino groups have been acylated and a number of reactions involving the interconversion of one tetrazolium salt to another have been described by Bamberger, Padova, and Ormerod (20). Amongst these should be mentioned one novel tetrazolium synthesis in which 3-nitro-1,5-diphenylformazan reacts with glacial acetic acid to give 5-hydroxy-2,3-diphenyltetrazolium betaine (table 28).



An attempt to quaternize the pyridyl nitrogen atom of 2,3-diphenyl-5-γ-pyridyltetrazolium iodide directly was unsuccessful. The corresponding formazan reacted smoothly with quaternizing agents (130).

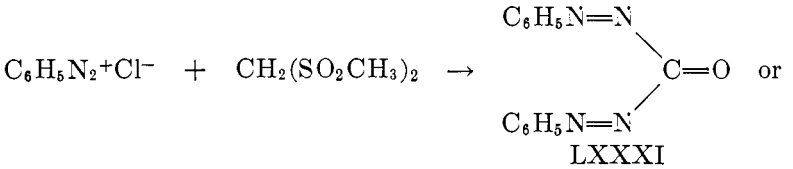
The preparation of a tetrazolium salt belonging to the isomeric series derived from (1*H*)tetrazole (page 357) has recently been described by Hünig and Boes (93). They condensed *p*-nitrobenzenediazonium borofluoride with dimethylsulfonylmethane; the main product was the expected hydrazone (LXXIX) but a smaller amount of a yellow solid was obtained, whose analysis and behavior suggested that it could be represented by LXXX, formed by the loss of two methanesulfonyl radicals.



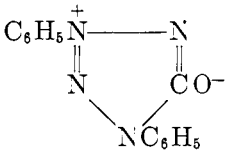
LXXIX

LXXX

LXXX is the betaine of the *C*-hydroxy(1*H*)tetrazolium compound, and Hünig and Boes have suggested that it has the same type of structure as the diphenyl compound (LXXXII) obtained by Backer (4) and formulated by him as the carbadiazone (LXXXI):

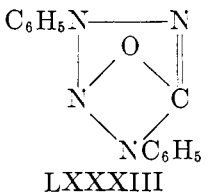


LXXXI



LXXXII

This product has different properties from those of the isomeric 5-hydroxy-2,3-diphenyltetrazolium betaine (CVIIIa, page 421) described by other workers. Hünig and Boes have shown that the compound prepared (together with several homologs) by Busch and Schmidt (47) and formulated as an endoxytetrazole (LXXXIII) had in fact the structure LXXXII.



LXXXIII

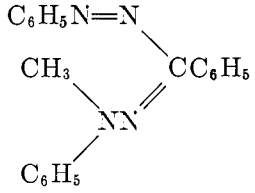
VIII. Physical Properties of Formazans

Formazans generally are solids of relatively low melting point for the size of the molecules. Very low melting or even liquid formazans apparently arise when a cholyl group is attached to the N^1 position (50), or when long chains are linked to the formazan nucleus (2). The triarylformazans as a whole (table 16) tend to melt in the region 155–215°C. Formazans are characterized by intense colors, ranging from orange through shades of red to a deep purplish black. At the same time, many show brilliant reflex colors and their appearance is often very striking. The influence of substituents on the color follows rules familiar in the dyestuffs field; the effect of nitro groups substituted in benzene rings has been described in detail (150). Formazans with aliphatic substituents (hydrogen, methyl, carboxyl, etc.) attached to the 3-carbon atom have lighter colors and a greater solubility in common organic solvents than have the aryl derivatives. The absorption spectra of formazans have been summarized in table 1 (page 374).

Triarylformazans are often particularly soluble in chloroform and acetone; in water the solubility appears to be negligible, the solvent being scarcely colored. As the molecule is loaded with larger groups such as diphenyl, styryl, phenylazo, etc., the color of the crystals becomes almost black and the solubility very low. Nitromethane is often an effective solvent in such cases. The only water-soluble formazans are those in which specifically solubilizing groups have been introduced into the molecule. These include the sulfophenyl compounds (table 16), the formazans containing quaternary groups (table 16), and structures like that derived from procaine (LXXVI) (2).

IX. Acid and Base Characteristics of Formazans

Formazans behave both as weak acids and as weak bases; both types of salt which can result are hydrolyzed by water in the cold. The deep red solutions which many formazans give with concentrated aqueous alkalies precipitate the free formazan upon dilution with water. Unstable solid sodium and potassium salts of characteristic bright colors, markedly different from those of the parent formazans, have been isolated (15, 17, 148). They are decomposed by damp air and instantly hydrolyzed by water. These salts react with methyl iodide to give *N*-methylformazans such as LXXXIV (table 10), which cannot be prepared by the direct method (45); the oxidation of these compounds is not described.



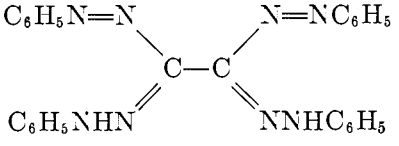
LXXXIV

Formazans substituted with specific acidic or basic groups show the behavior characteristic of these groups. Those bearing a sulfonic acid group have only been isolated as sodium and potassium salts, since these can usually be crystal-

lized from water or alcohol (66). 3-Nitro-1,5-diphenylformazan shows a strongly acid reaction, due to the ionization of the pseudo-nitro form (7).

Formazans generally form metallic complex "salts," particularly with copper, nickel, cobalt, and silver. A few of these have been studied in detail (94) and their properties adduced as evidence for the chelated mesomeric character of formazans. These properties are typical of those of chelated complexes, for the salts are readily soluble in such solvents as chloroform, benzene, and acetone, and have low, sharp melting points and deep clear colors. The stability of these complexes depends largely on the nature of the substituents in the aromatic rings. The silver salt of 3-benzoyl-1,5-diphenylformazan (table 13) is very unstable (27, 28). The copper salts of formazans whose *N*-phenyl rings carry hydroxyl or carboxyl groups in the ortho position are particularly stable (56, 168, 199, 201); they are not hydrolyzed by acids and have been used for dyeing. The stability of this class of complex can be attributed to chelation involving the hydroxylic ortho substituents.

Salts of formazans with acids have not been isolated in a pure state (except where there is a basic substituent such as a quaternary group present). Formazan solutions become much darker in color upon the addition of mineral acids, and some water-insoluble formazans dissolve in aqueous mineral acids. The dark solutions apparently contain formazan cations, but these solutions are hydrolyzed on dilution and the free formazan is precipitated. The instability of formazans to acid hydrolysis (see page 416) makes the investigation of these salts difficult. It is surprising that 3,3′-(1,5-diphenyldiformazyl) (LXXXV, table 20) or "diformazyl" is a strong base, and that salts such as the sulfate and hydrochloride have been prepared in the solid state (16, 19).

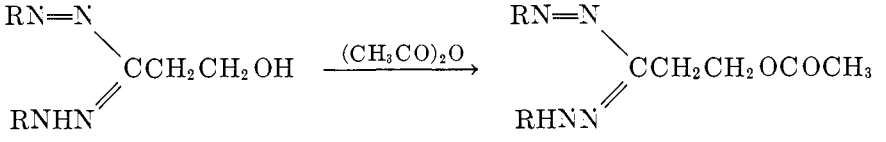


LXXXV

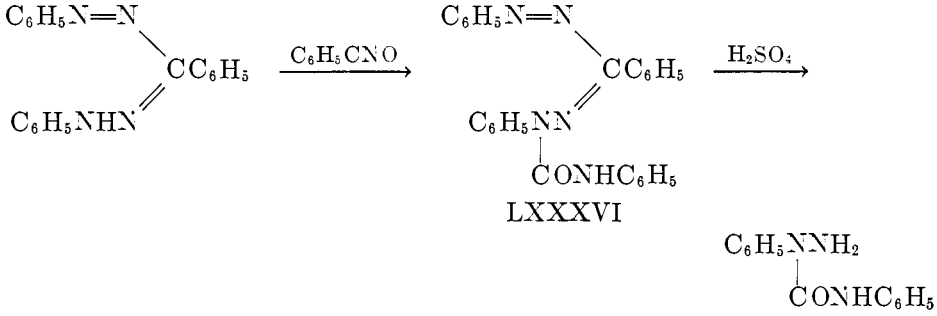
X. Reactions of Formazans

A. Acylation

Formazans cannot be acylated very readily. Attempts to benzoylate triphenylformazan with benzoyl chloride (42) or to acetylate 3-methyl-1,5-diphenylformazan and other formazans with acetic anhydride (15, 60a) have been unsuccessful. Acetylation can be accomplished with acetic anhydride in the presence of zinc chloride (15, 19, 28, 132), and can be achieved in special cases by the use of acetic anhydride alone (see page 365). *C*-Hydroxyalkylformazans have been found to react with warm acetic anhydride to give the *O*-acetyl derivatives only (60a).



N-Acylation "fixes" the tautomeric system of the formazans, giving, in the case of unsymmetrical formazans, a mixture of the two possible isomeric *N*-acetylformazans, described on page 365. Reaction with phenyl isocyanate has been reported (41), yielding *N*-carbanilidoformazans (LXXXVI). These undergo hydrolysis when treated with sulfuric acid to give 2,4-diarylsemicarbazides, which are most readily accessible in this way:

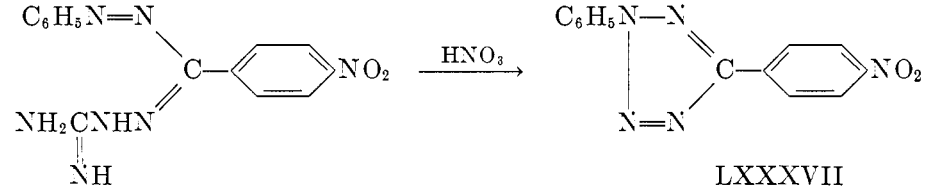


LXXXVI

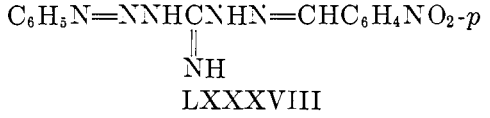
B. OXIDATION

The oxidation of formazans to tetrazolium salts has already been discussed (page 403) under the section on the preparation of the latter. This specific oxidation occurs with particular oxidizing agents; the use of more powerful ones (e.g., concentrated nitric acid) causes complete destruction of the molecule.

The guanazyls (page 401) undergo special oxidative reactions with concentrated nitric acid to form disubstituted tetrazoles (183). This observation constitutes evidence that the guanazyls have the structure exemplified by LXXXVII and not the isomeric azo structure (LXXXVIII):

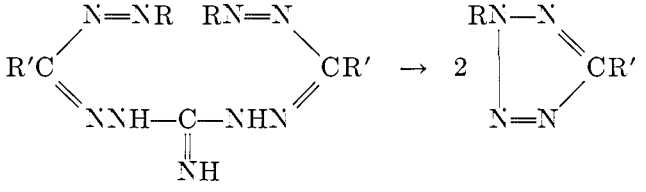


LXXXVII

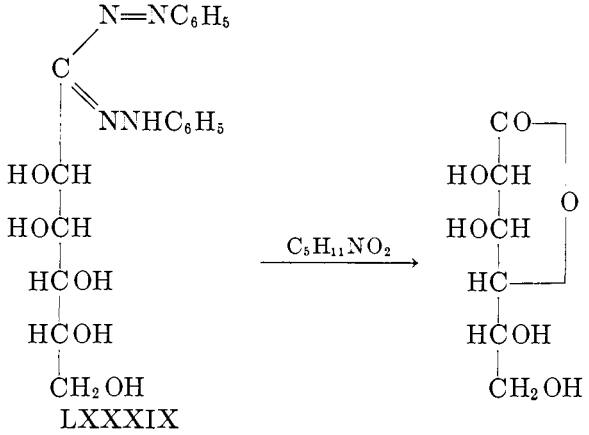


LXXXVIII

Concomitant nitration of aromatic rings is sometimes observed (162, 163). Oxidation of the guanazyls proceeds smoothly, and in an analogous manner, with lead tetraacetate. This reaction has recently been applied (161, 162, 163) to the synthesis of 2,4-diaryltetrazoles from bisguanazyls which lose the guanidine carbon atom in the same way as monoguanazyls during the oxidation:



Formazans carrying a sugar residue undergo abnormal oxidation with isoamyl nitrite and hydrogen chloride, the product in the case of 3-*d*-manno-1,5-diphenylformazan (LXXXIX) being *d*-mannonic acid γ-lactone.



LXXXIX

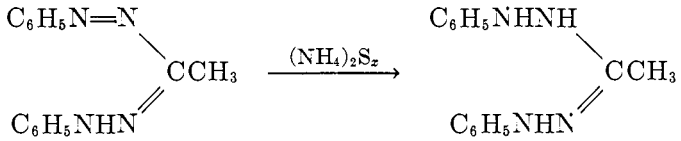
Other oxidizing agents did not give simple products. The corresponding tetrazolium salts were obtained by a preliminary protection of the hydroxyl groups by means of acetylation, followed by oxidation by lead tetraacetate (205).

C. REDUCTION

Compounds of several types are produced by the reduction of formazans.

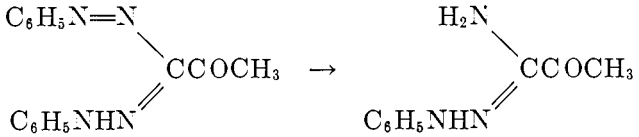
1. Ammonium sulfide

The relatively mild action of ammonium sulfide leads to a discharge of color and the reduction of the azo group to a hydrazo group (176).



XC

These products are called *hydrazidines*[6] (XC). Like most hydrazo compounds, hydrazidines are unstable in oxidative conditions and readily revert to formazans. In most cases, however, this type of reduction proceeds further, an arylamine molecule being split off and an *N*-arylamidrazone (XCI) formed (13, 14, 15, 27, 28, 176).

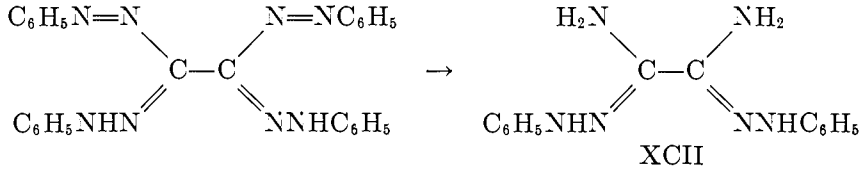


XCI

"Diformazyl" likewise gives "diamidrazone" (XCII) (16, 19).

[6] Unfortunately, this name has been applied to other molecular structures. See the report on nomenclature in *Chemical Abstracts* (128).

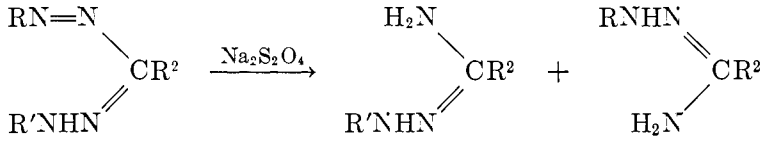
The reaction between ammonium sulfide and 3-nitro-1,5-diphenylformazan follows a different course, the product being 3-mercapto-1,5-diphenylformazan (20).



XCII

2. Sodium dithionite

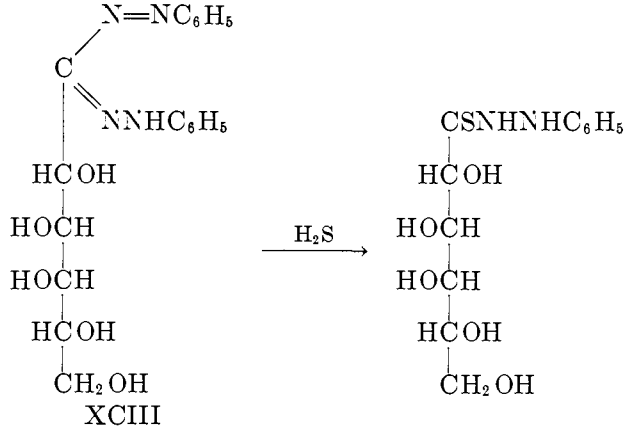
This reagent in aqueous solution also reduces formazans, usually dissolved in acetone, to *N*-arylamidrazones (101). The two *N*-aryl substituents in the formazan reduced should be the same (the so-called symmetrical formazans) if the amidrazones are to be isolated; unsymmetrical formazans, as a result of their mesomerism, give a mixture of two amidrazones which would probably be exceedingly difficult to separate.



The formazans derived from diarylsemicarbazones (155) (table 25; see pp. 402, 461) are reduced to colorless compounds by sodium dithionite solution. These are apparently hydrazidines, since the red color is rapidly restored in air; no pure reduction products could be isolated. The amidrazones have attracted some interest of late because simple types recently studied (101) showed a weak tuberculostatic and antifungal activity.

3. Hydrogen sulfide

The sugar-formazans recently described by Hungarian workers undergo partial fission when treated with alcoholic hydrogen sulfide. Decolorization of 3-*d*-galacto-1,5-diphenylformazan (XCIII) leads to thiogalactonic acid phenylhydrazone (206).



XCIII

4. Catalytic hydrogenation

Catalytic hydrogenation of formazans using palladized barium sulfate leads in turn to hydrazidines and amidrazones (102). The reaction can be stopped at the hydrazidine stage (when one molecule of hydrogen has been taken up). The hydrazidine is stable in the case of *C*-alkylformazans (table 10) and 3-methyl-1,5-diphenylformazan gives β,β-*N*,*N*-diphenylacethydrazidine (XC). This has also been produced by the action of aqueous sodium dithionite (102).

The diarylsemicarbazone-derived formazans mentioned above also gave hydrazidines when palladized charcoal was used as catalyst (155). In the presence of Raney nickel, four hydrogen atoms were taken up and the amidrazone was apparently formed. The amidrazone (XCIV) is the stable product obtained during the hydrogenation of 1,3,5-triphenylformazan (102).

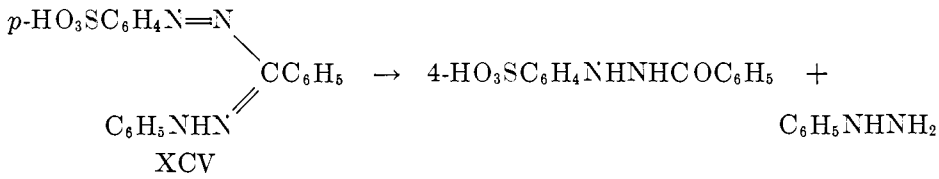
$$C_6H_5C(NH_2)=NNHC_6H_5$$

XCIV

5. Zinc and dilute acids

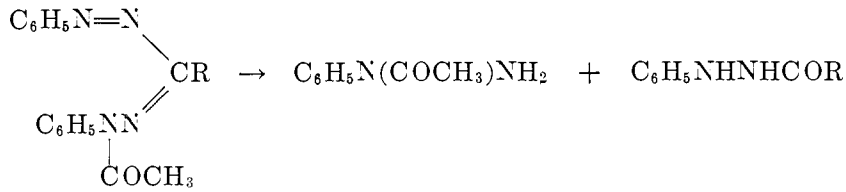
Zinc and dilute sulfuric acid reduce formazans to a mixture of a hydrazine and an acylhydrazide. When unsymmetrical formazans (bearing dissimilar *N*-aryl groups) are reduced in this way, the two hydrazines and the two acylhydrazides expected from the two tautomeric forms are produced (42, 139; see page 366).

1,3-Diphenyl-5-*p*-sulfophenylformazan (XCV, table 16) is anomalous in this respect. Only phenylhydrazine and β-benzoyl-*p*-sulfophenylhydrazide can be isolated from the reduction by zinc and acid.



XCV

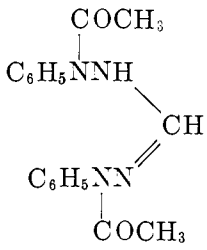
When the tautomerism is fixed by acetylation, the reduction of the acetylformazan gives the expected single pair of products, one being in this case an α-acetylphenylhydrazine:



6. Zinc dust

Reduction with zinc dust alone leads to a complete breakdown of the formazan molecule to unidentified simple products (134).

Zinc dust and acetic anhydride effect concomitant reduction and acetylation to a diacetylhydrazidine such as XCVI (132).

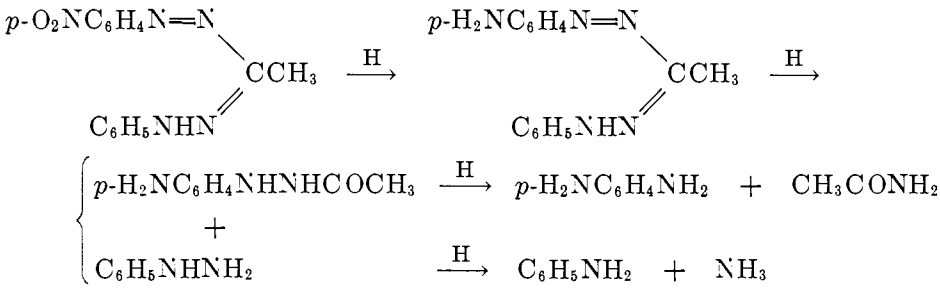


XCVI

7. Stannous chloride

Reduction with stannous chloride can be controlled so that aromatic nitro groups are reduced to amino groups without the formazan molecule being affected (131, 181). This has been achieved in the guanazyl series, and with certain formazans with a 2,4-dinitrophenyl group attached to carbon, which are peculiarly resistant to acid hydrolysis.

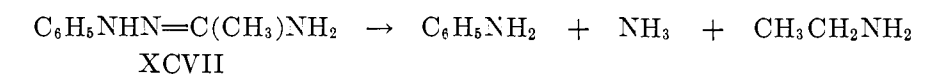
Most formazans, however, undergo further reduction with stannous chloride with the formation of amines, hydrazines, and acids. This reaction has been used by several workers to establish the structure of formazans (83, 147, 149). The reduction was studied in detail by Ragno and Bruno (149), who postulated the following mechanism:



They also showed that this reduction offered a further confirmation of the mesomeric character of the formazan molecule, by an elaboration of the earlier arguments put forward by von Pechmann.

8. Other reducing agents

Sodium amalgam splits the 1,3,5-triphenylformazan molecule to give benzaldehyde phenylhydrazone (102). Formazans and the derived amidrazones are reduced further by hydriodic acid to give ammonia and amines. Thus, *N*-phenylacetamidrazone (XCVII) yields ammonia, aniline, and *n*-propylamine in such circumstances (97).

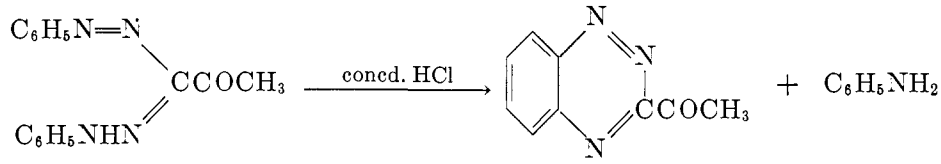


XCVII

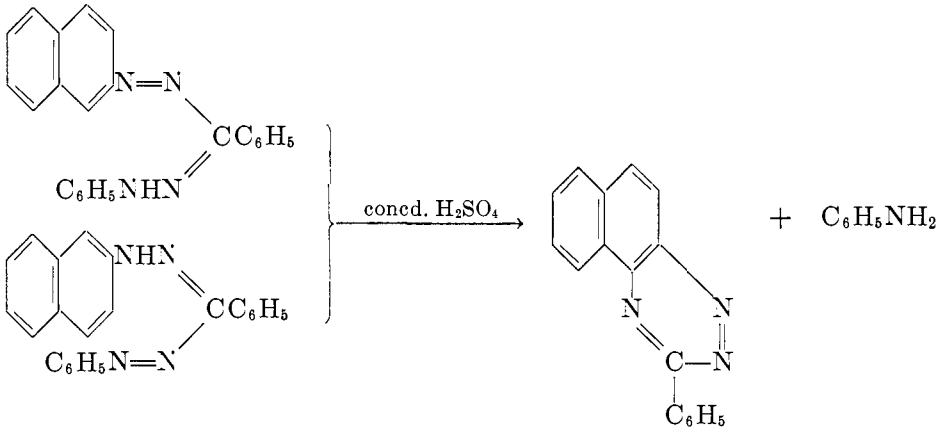
D. ACTION OF ACIDS

Many formazans are readily decomposed by acids. They dissolve in cold concentrated sulfuric acid to give characteristic intensely colored solutions. Bamberger and Wulz (29) purified 3-acetyl-1,5-diphenylformazan by dissolving it under these conditions and reprecipitating it unchanged with ice. Very little sulfonation was observed.

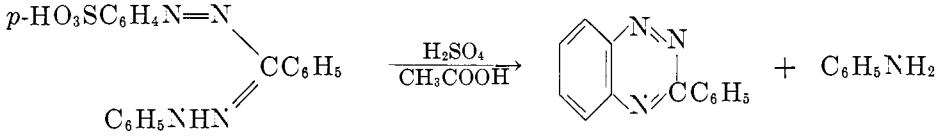
Mineral acids at slightly higher temperatures cause formazans to rearrange to benztriazines (15, 17, 26, 27, 28, 134). Thus,



The two tautomeric forms of unsymmetrical formazans both rearrange to the same product. Fichter and Schiess (66) were unable to distinguish between the supposedly different forms of 1-β-naphthyl-3,5-diphenylformazan prepared by the two possible alternative routes:

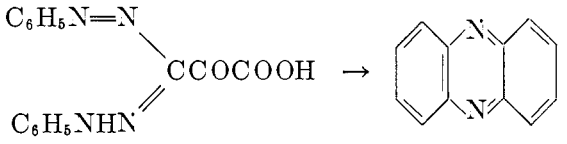


No 2-β-naphthylbenztriazine was detected. How the different aryl groups in an unsymmetrical formazan determine which of the two possible benztriazines is formed in this rearrangement is not known. 1,3-Diphenyl-5-*p*-sulfophenylformazan gives aniline and phenylbenztriazine; the sulfonic acid group is apparently lost in this process (66).

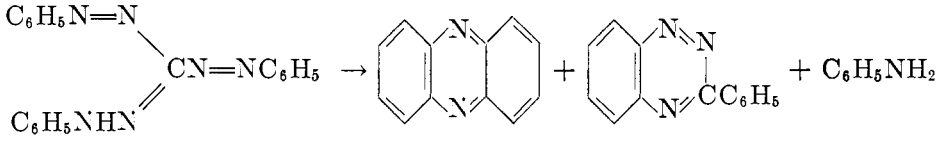


In the case of 3-*o*-hydroxyphenyl-1,5-diphenylformazan (table 16) the rearrangement to the anticipated 3-*o*-hydroxyphenylbenztriazine can be accom-

plished with hot glacial acetic acid as well as with concentrated sulfuric acid. The so-called "formazylglyoxylic acid" (XCVIII, table 13) rearranges with concentrated hydrochloric acid to phenazine (XCIX), whilst 1,5-diphenyl-3-phenylazoformazan (C) gives both phenazine and α-phenylbenztriazine (CI):



XCVIII XCIX

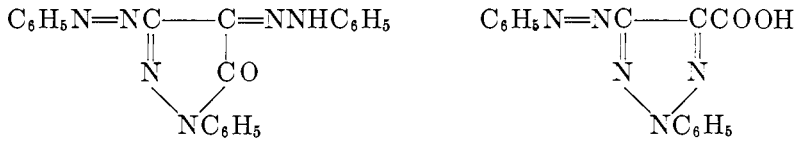


C CI

The reaction of 3-methyl-1,5-diphenylformazan, in acetic acid solution, with warm concentrated sulfuric acid yields phenazine as the main product, accompanied by traces of 2-methylbenztriazine (129a). It seems possible that the rearrangement to a phenazine is more likely than to a benztriazine except when an aryl group is attached to the formazan carbon atom.

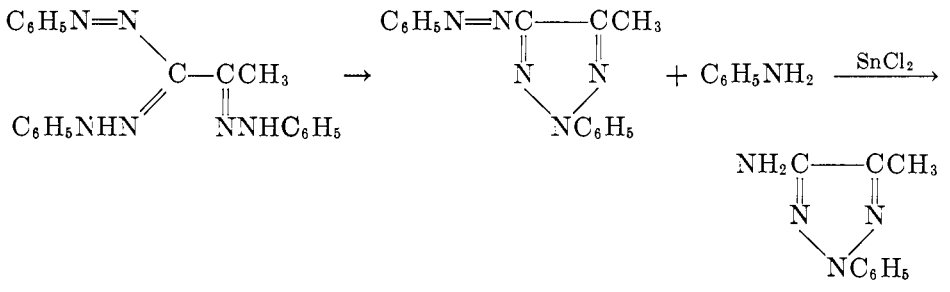
Upon treatment with cold hydrochloric acid, 3-methyl-1,5-diphenylformazan is said to suffer fission to phenylhydrazine and benzenediazonium chloride (11).

The phenylhydrazone of "formazylglyoxylic acid," which was not isolated in a pure form, was shown to undergo rearrangement in acetic acid to CII and CIII (18, 19):

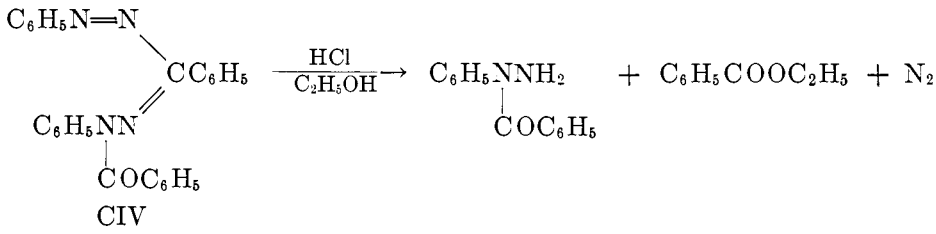


CII CIII

A product analogous to CIII is reported (15, 17) as being formed in the rearrangement of 3-acetyl-1,5-diphenylformazan phenylhydrazone upon boiling in acetic acid solution:



The less soluble triarylformazans are more resistant to acid hydrolysis; however, Wedekind was unable to isolate the free amino compound from the action of warm hydrochloric acid on 1-*p*-acetamidophenyl-3,5-diphenylformazan (187). Alcoholic hydrogen chloride causes the complete breakdown of 1-benzoyl-1,3,5-triphenylformazan (CIV) as shown:



CIV

The solubility of the formazan influences its stability to acids to a great extent. 3-(2′,4′-Dinitrophenyl)-1,5-diphenylformazan (table 16) is apparently unaffected by boiling concentrated hydrochloric acid (131), but after reduction with stannous chloride to the diaminoformazan, it is readily converted to α-(2,4-diaminophenyl)benztriazine.

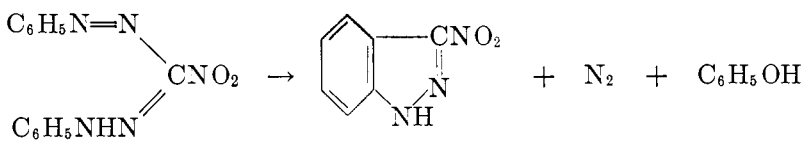
The complex heavy metal salts of formazans are, in general, readily hydrolyzed by acids. On the other hand, salts of this type in which the *N*-phenyl rings carry carboxyl or hydroxyl groups in the ortho position, were found by Wizinger and Biro (199) to possess remarkable stability in acid solutions.

The decomposition of 1,5-diphenylformazan-3-sulfonic acid is especially facile. This substance is readily decomposed by boiling with dilute acids (136); it is also rapidly reduced.

E. ACTION OF ALKALIES

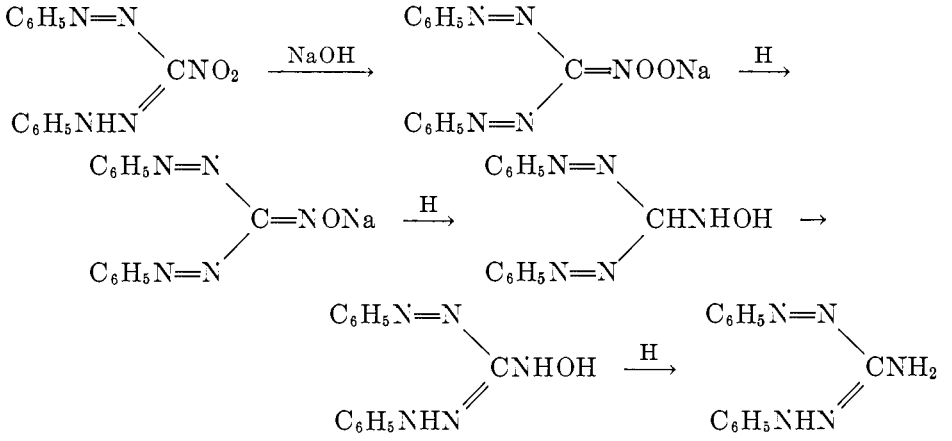
Alkalies do not easily cause a break-up of the formazan molecule and can therefore be used for the hydrolysis of substituent groups, leaving the basic structure unaffected. Thus, 3-cyano-1,5-diphenylformazan (table 13) has been converted to the 3-carboxylic acid by heating it with the calculated amount of potassium hydroxide solution for 1.5 hr. (193).

3-Nitro-1,5-diphenylformazan is hydrolyzed in alkaline conditions to the corresponding 3-hydroxyformazan, which is oxidized rapidly to 5-hydroxy-2,3-diphenyltetrazolium betaine (8, 20). In a mixture of acetic acid and concentrated sulfuric acid this 3-nitroformazan is cyclized, with loss of nitrogen, to 3-nitroindazole (20).



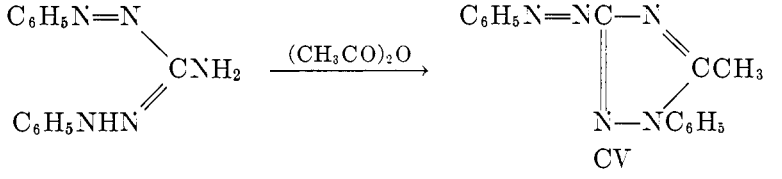
The same formazan undergoes an unusual interchange in sodium hydroxide solution when treated with sodium hydrosulfide. The product is 3-amino-1,5-

diphenylformazan (table 12) (whereas ammonium polysulfide gives the 3-mercapto compound). The reactions involved are apparently as follows (20):



The mercaptoformazan is formed as a by-product.

3-Amino-1,5-diphenylformazan displays anomalies in its behavior; it cannot be formylated or benzoylated by normal techniques, whilst the action of acetic anhydride gives rise to 5-methyl-1-phenyl-3-phenylazotriazole (CV).

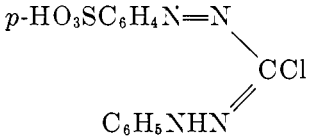


The formazan is a weak base, the hydrochloride being hydrolyzed by water. Concentrated hydrochloric acid does not promote the usual rearrangement to a benztriazine; instead, one benzene ring is chlorinated. Hydrogen bromide similarly effects monobromination of the formazan in ethereal solution (20).

F. OTHER REACTIONS

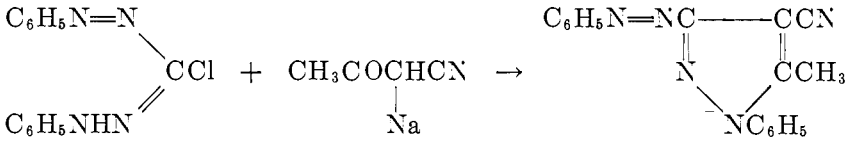
The formazyl halides (e.g., CVI, table 12) described by Fusco and Romani (74, 75) can behave either as formazans, or as halide-hydrazones, compounds which possess a very reactive halogen atom. The chlorine can readily be replaced by iodine by the normal Finkelstein reaction. The *C*-bromoformazans could not be prepared in this way (see page 400). Attempts to replace the chlorine atom by OH, $OCOCH_3$, OCH_3, and NO_2 were unsuccessful, nitrogen being lost and tars produced. Silver nitrate in aqueous acetone solution precipitated silver chloride, but no other product was isolated. Reaction with alcoholic ammonia solution gave the *C*-aminoformazan, and sodium hydrosulfide gave the *C*-mercapto compound. Oxidation with isoamyl nitrite led to 5-hydroxy-2,3-diphenyltetrazolium betaine; reduction with stannous chloride gave aniline hydrochloride and β-carbamylphenylhydrazine (74). Sulfonation, achieved by

dissolving the chloroformazan in cold concentrated sulfuric acid, gave CVI, a dark lustrous-blue solid dissolving in water to a yellow solution.



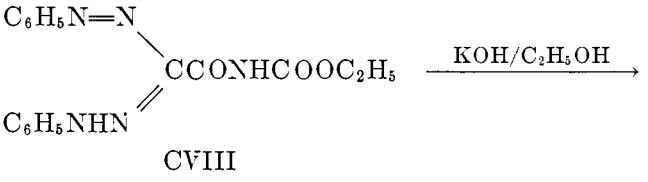
CVI

Another interesting reaction of these compounds is their condensation with the sodium derivative of cyanoacetone to form pyrazoles (CVII):

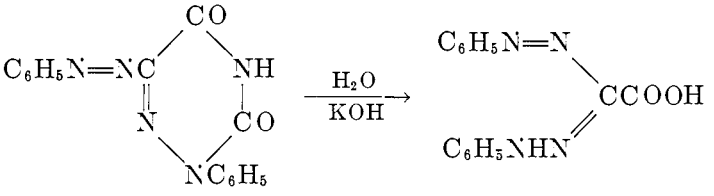


CVII

Several formazans have been described in which a urethan group is attached at the 3-position (CVIII, table 13) (195). These undergo ring-closure in alcoholic alkali, but aqueous alkalies reopen the ring, to give the formazan-3-carboxylic acid:



CVIII



G. ACTION OF LIGHT

The action of visible light on solutions of formazans, with its relation to formazan structure, has been discussed on page 369. Ultraviolet light decolorizes formazan solutions; triphenylformazan behaves in the same way as its corresponding tetrazolium salt and is converted to the bicyclic tetrazolium salt (CX) (page 424) (80).

XI. Physical Properties of Tetrazolium Salts

The general properties of tetrazolium salts were first summarized by von Pechmann and Runge (140). The free bases are powerfully alkaline and, like other quaternary bases, may be generated from halide salts by means of moist

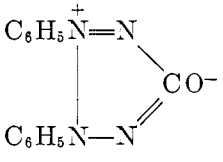
silver oxide. The simpler members of the class are water-soluble, absorb carbon dioxide from the atmosphere, dissolve zinc hydroxide (139), and in general behave like the fixed alkalies towards metallic salts. Tetrazolium hydroxides are unstable, cannot be isolated as solids, and are readily reduced.

The corresponding salts in general are stable and crystalline and are formed with weak acids. Many of these salts, in particular the chlorides, which are the best known, are soluble in water and give solutions of neutral pH. This solubility is high in the simpler members but diminishes with increasing substitution in the aromatic rings until the chlorides of the azo- and styryltetrazolium salts (tables 30 and 31) are found to have very slight water-solubility. All these chlorides are readily soluble in methanol and ethanol. The bromides and iodides are progressively less soluble in these solvents. Salts of strong acids, such as nitric and perchloric acids, are relatively insoluble even in simple cases.

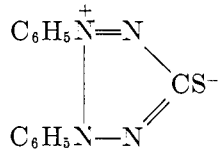
Other difficultly soluble salts of sharp melting point which have been prepared from triphenyltetrazolium chloride are the thiocyanate (m.p. 134–136°C.), the picrate (m.p. 186–188°C.), and the mercuric chloride compound (m.p. 235–237°C.) (100). Even in the case of the tetrazolium salts bearing trimethylammonium substituents (table 29), in which the chlorides and methyl sulfates are highly water-soluble, the perchlorates are but sparingly soluble. The simpler tetrazolium salts are colorless (unless the anion confers color) and possess a bitter taste. They often become yellow in bright light (see page 423).

A few absorption spectra of tetrazolium salts have been measured (81, 106, 163a). Weak absorption maxima were shown at 260–290 mμ for 5-hexyl-2,3-diphenyltetrazolium chloride and at 250 mμ for triphenyltetrazolium chloride (81) (T.T.C.). Several ditetrazolium salts showed absorption peaks between 240 and 260 mμ (163a).

Grammaticakis (77) has measured the absorption of the so-called diphenylcarbodiazone and diphenylthiocarbodiazone, which Bamberger has convincingly shown to be in the tetrazolium betaine form (9) (table 28); he has obtained results confirming that these salts should be represented by CVIIIa and CVIIIb and not by the open-chain formulas originally postulated.



CVIIIa



CVIIIb

For a discussion of polarographic studies and reduction potentials of tetrazolium salts, see page 422.

XII. Reactions of Tetrazolium Salts

A. Reduction

Reduction is the most characteristic reaction of tetrazolium salts, and one which can be achieved in a variety of ways. Although powerful reducing agents

break the molecule down to small fragments, the use of milder agents leads to formazans. Such reduction by chemical means only appears to take place at a pH above 7; biological reductions are discussed later.

Ammonium sulfide (180) was employed in earlier work, although this reagent will further reduce a formazan in solution; if the formazan is precipitated from aqueous solution, further reduction is prevented. Stepwise catalytic hydrogenation has been described by Jerchel and Kuhn (102): formazans are formed at the first stage; the further reduction of these primary products has already been discussed (page 414). These authors also investigated the use of sodium amalgam and sodium dithionite as reducing agents. Ascorbic acid (in alkaline solution) effects a smooth clean reduction, and has been used recently in the preparation of a highly purified sample of 1,3,5-triphenylformazan from triphenyltetrazolium chloride (109). Reductone gives a pale rose color with triphenyltetrazolium chloride solution at pH 5.15, but a dark red precipitate of formazan is produced at pH 10.7 (62). The author has found alkaline solutions of hydroxylamine and hydrazine to be valuable reducing agents which have the advantage of forming completely innocuous by-products; the former reagent has recently been used to prepare a formazan (126).

The reduction potentials of tetrazolium salts have been measured (103, 157) and their significance in biochemistry discussed (see page 428). Such measurements have been used to demonstrate a stepwise reduction (comparable with that obtained by catalytic hydrogenation) and have made it possible to arrange different tetrazolium salts according to their reduction potentials. Jerchel and Möhle measured the reduction potentials of some tetrazolium salts by an indicator method, assuming that the system was

$$\text{Tetrazolium salt} \xrightarrow{2\epsilon} \text{formazan}$$

They reported a value on the hydrogen electrode scale of -0.08 v., independent of pH, for triphenyltetrazolium chloride. A detailed polarographic study of this substance over the pH range 2–12 has been made (48a), and two waves, due to a 4 electrons per molecule and a 2 electrons per molecule reduction, were obtained. Over the pH range 7–9, a third 2 electrons per molecule reduction wave was observed. An attempt has been made to correlate half-wave potential with structure and with bacteriostatic activity for a number of substituted tetrazolium salts. Polarographic studies by Ried and Wilk (157) showed that the current–potential curve is characteristic for particular classes of salts (e.g., bistetrazolium salts), although this work was carried out in unbuffered solutions.

The simple tetrazolium salt 5-hydroxy-2,3-diphenyltetrazolium betaine (CVIIIa, table 28) is exceptionally readily reduced. Boiling in dilute sodium hydroxide solution breaks down the molecule to benzene, aniline, biphenyl, and traces of other products (20).

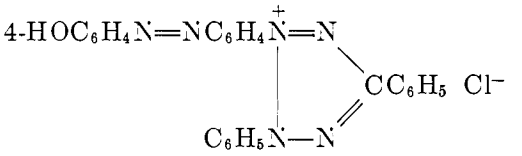
B. OXIDATION

Tetrazolium salts are resistant to oxidation. In their attempts to degrade a tetrazolium salt to tetrazole, as a proof of constitution, von Pechmann and Wedekind (141, 180) found it necessary to introduce hydroxyl groups into the

benzene rings to make possible potassium permanganate oxidation to tetrazole (see page 378). The reaction has not been otherwise studied.

C. ACTION OF ACIDS

Tetrazolium salts are remarkably stable to acids. In this, they show a marked contrast to the formazans, which are readily broken down by warm dilute acids. This stability is exemplified by the preparation of mono- and dihydroxytetrazolium salts by the fission of alkoxyl groups with concentrated halogen acids at 140–150°C. (141, 180), by the conversion of acetamido- to aminophenyltetrazolium salts by prolonged reflux with concentrated hydrochloric acid in the presence or absence of alcohol (2), and by the behavior of tetrazolium compounds with sulfuric acid. Hot concentrated sulfuric acid merely effects incomplete sulfonation, although charring results at still higher temperatures. 3-(4′-Hydroxyphenylazophenyl)-2,5-diphenyltetrazolium chloride (CIX) was unchanged after heating with concentrated sulfuric acid at 100°C. for 2 hr. (126a).

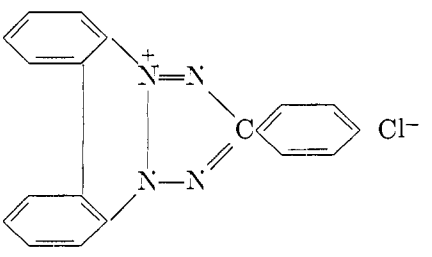


CIX

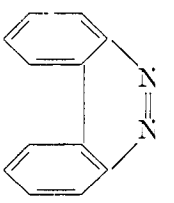
Treatment of triphenyltetrazolium chloride with 95 per cent oleum at room temperature causes disulfonation, but the points of reaction have not been ascertained (126a).

D. ACTION OF LIGHT

Many, if not all, tetrazolium salts are sensitive to light, and freshly prepared samples of colorless crystals turn yellow at a rate depending upon the structure of the compound. This change, which becomes visible in a few hours, may occur with colored tetrazolium salts, but it has only been investigated in detail in the case of triphenyltetrazolium chloride. The action of ultraviolet light (and, to a lesser degree, of ordinary light) on an aqueous solution of this compound produces two new products: (*1*) the corresponding formazan (which explains the development of a red color), and (*2*) the oxidation product (CX), called 2,3-(2,2′-diphenylene)-5-phenyltetrazolium chloride or "Photo T.T.C." (81, 194, table 35).



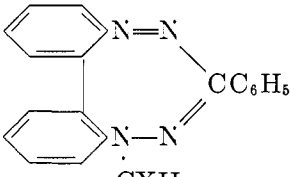
CX



CXI

This forms colorless needles with bright blue fluorescence. It is the sole product if the irradiation is carried out in ethanolic solution. The somewhat unexpected structure (CX) was confirmed by reduction with zinc and hydrochloric acid, which gave phenazone (CXI), and by distillation with zinc dust, which gave benzonitrile and carbazole (81, 194). Triphenylformazan in ethanolic solution is also converted by ultraviolet light into the compound CX with decolorization. The quantitative aspects of this effect of light were described by Hausser (80).

When "Photo T.T.C." is reduced with stannous chloride in aqueous solution in the presence of benzene, an olive-green solid is obtained (81, 107). This is also produced by the reduction of "Photo T.T.C." with potassium in liquid ammonia, sodium dithionite in sodium bicarbonate solution, or sodium hypophosphite in water. The reactive product is apparently a free radical (one mesomeric form of which is CXII) which is stable to air in benzene solution and is rapidly decolorized by ultraviolet light and by acetic acid, aqueous hydrochloric acid, alcohol, and iodine. The absorption spectrum (showing maxima at 290, 335, 400, and 515 mμ) and paramagnetism of CXII have been measured.



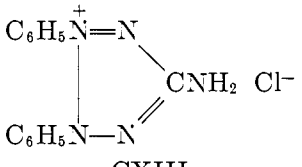
CXII

An attempt to produce the corresponding bicyclic compound from 2,5-diphenyl-3-*p*-styrylphenyltetrazolium chloride (table 30) by irradiation led only to the recovery of unchanged starting material (129a).

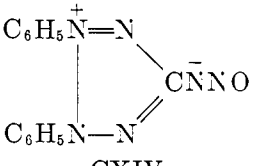
E. OTHER REACTIONS

Tetrazolium salts with substituent amino groups (table 29) have been reported, in certain cases, to form well-defined crystalline mercuric chloride complexes. Such complexes may be produced when mercuric oxide is used for the oxidation of acetamidoformazans (2). Lead tetraacetate is the reagent of choice for the oxidation of formazans of this type, since isoamyl nitrite and hydrogen chloride have been found in some cases to cause hydrolysis of the acetamido group, followed by diazotization of the free amino group produced. The diazotized material then decomposed during the working-up, with loss of nitrogen, so that the aminotetrazolium salt was contaminated with appreciable amounts of deaminated product. The separation of such a mixture was impracticable (126).

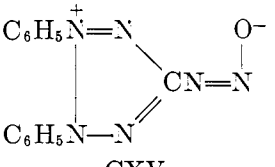
The simplest aminotetrazolium salt known is 5-amino-2,3-diphenyltetrazolium chloride (CXIII, table 28):



CXIII



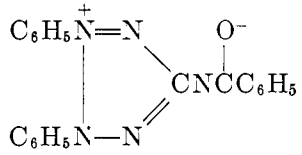
CXIV



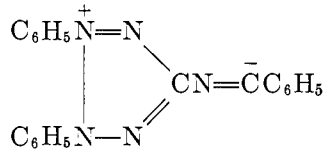
CXV

Nitrous acid reacts with this to form an *N*-nitrosobetaine (CXIV), which reverts to CXIII on warming with dilute hydrochloric acid, but which rearranges in boiling ethanol to the isomeric betaine (CXV) (20).

The benzoylation of CXIII leads to the betaine CXVI or its hydrochloride, and this compound can be reduced to the betaine CXVII (20):



CXVI



CXVII

XIII. Uses of Formazans

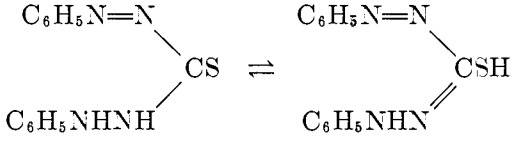
The application of formazans to purposes other than synthesis has been limited.

A. DYESTUFFS

Fichter and Schiess (66) prepared several formazans with substituent sulfonic acid groups, as their sodium or potassium salts, and studied their potentialities as dyestuffs. This use of formazans does not appear to have been exploited until Ciba Ltd. claimed a patent in 1946 (56). This makes use of the dyeing properties of the copper complex salts of formazans with *o*-hydroxyl and *o*-carboxyl groups on the *N*-phenyl rings. These salts are more stable than the free formazans, especially towards acids; they give very intense colors in aqueous solution and are fast wool dyes. The patent mentions a large variety of these compounds, but they are not described or enumerated in detail (see also 199).

B. METALLIC REAGENTS

One class of formazans (table 12), derivatives of the so-called diphenylthiocarbazone (CXVIII) ("dithizone"), has been used as reagents for various heavy metals (96, 129):



CXVIII

Among several such compounds which have been used as reagents for traces of metals, di-β-naphthylthiocarbazone (3-mercapto-1,5-di-β-naphthylformazan) has been used to detect traces of mercury and zinc in biological materials. The preparations of this reagent and several homologs, and their transmission curves in light of various wave lengths, have been described by Hubbard and Scott (92). The preparation of dithizone itself has been described in detail by Billmann and Cleland (35) and, very recently, Irving and Bell (96) have compared the various synthetic routes to this compound. The latter workers were interested in the preparation of dithizone containing the ^{35}S isotope for studies on the partition

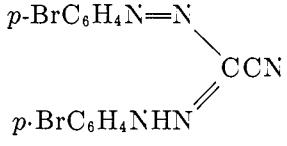
equilibria governing the extraction of traces of metals. They found that the preparation of the rigorously pure material could be effected in 33.3–39.8 per cent yields through the 3-chloro-1,5-diphenylformazan, but that analogs such as the di-β-naphthyl compound (*vide supra*) could be obtained only in 2.5–2.8 per cent yields. These authors also showed that the metallic derivatives are *S*-linked and not *N*-linked, since the *S*-methyl compound does not form metallic salts (95).

The oxygen analog of dithizone behaves in a similar way (51, 52) and forms an insoluble silver "salt" in the presence of slight traces of silver ions. Such "salts" are immediately decomposed by acids. This compound does not form insoluble salts with alkaloids.

3-Carboxy-1,5-diphenylformazan will detect 1 part of silver ions in 136,000 parts of solution and has been suggested as a reagent for silver (110), since most of the common metals do not interfere with the test.

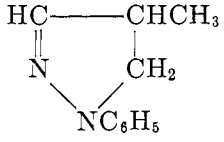
C. CHEMICAL USES

In the sphere of pure chemistry, Borsche and Manteuffel (37) carried out experiments on the condensation of aliphatic nitriles with esters and identified intermediates of the type of cyanoacetaldehyde by converting them to formazans. Thus, with 4-bromobenzenediazonium chloride, the reaction mixture in which cyanoacetaldehyde was thought to be present as an intermediate gave the formazan CXIX:

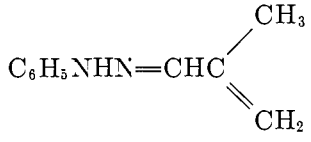


CXIX

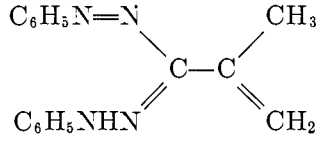
Duffin and Kendall (60) established that the compound previously thought to be the pyrazoline (CXIXa) was in fact α-methylacraldehyde phenylhydrazone (CXIXb), since it could be condensed with benzenediazonium chloride in alkaline solution to give 3-isopropenyl-1,5-diphenylformazan (CXIXa).



CXIXa



CXIXb



CXIXc

These authors also showed that, in alkaline media, pyrazolines of the type of CXIXa do not condense with diazonium salts to give formazans.

A patent has been issued (73) for the use of 3-methyl-1,5-diphenylformazan in waxes and polishes.

No studies of the biological or chemotherapeutic activity of formazans have been described, probably because of the generally insoluble character of formazans in suitable solvents, especially water. Recently, formazans whose aromatic

TABLE 4

Bacteriostatic action of tetrazolium salts

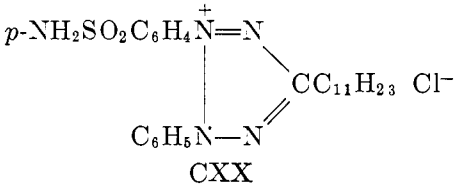
Tetrazolium Chloride	Lactic Acid Bacillus		Staphylococcus	
	Complete inhibition of growth	Unhindered growth	Minimum lethal dose	Unhindered growth
2,3-Diphenyl-5-undecyl-	1:400,000	1:1,100,000	1:38,400	1:409,600
3-α-Naphthyl-2-phenyl-5-undecyl-	1:300,000	1:1,500,000	1:38,400	1:76,800
2-*p*-Bromophenyl-3-phenyl-5-undecyl-	1:200,000	1:1,400,000	1:2,400	1:4,800
2-Phenyl-3-*p*-sulfonamidophenyl-5-undecyl-	1:200,000	1:1,500,000	1:400	1:3,200

rings contain quaternary ammonium substituents and which are somewhat soluble in water have been reported (126); the biological properties of these compounds have not yet been described.

XIV. Uses of Tetrazolium Salts

A. APPLICATION TO BIOLOGICAL RESEARCH

The first recorded application of tetrazolium salts to other research problems was that of Kuhn and Jerchel (106), who in 1941 included a study of tetrazolium salts in a program of research on "invert soaps." After discovering that triazolium salts possessed a notable disinfectant action, they looked for a similar property in tetrazolium salts. They were able to report a distinct antibacterial action in certain cases, in which the 5(carbon)-position of the tetrazole ring carried an *n*-undecyl group. The activity (see table 4) was of the same order as that of "zephirol" (benzyllauryldimethylammonium bromide). They also observed that compounds of this type, bearing a long alkyl chain, precipitated albumin in solutions with a pH greater than that of the isoelectric point of proteins. Subsequent experiments with similar tetrazolium salts, which also carried a *p*-sulfonamido group in one *N*-phenyl group (e.g., CXX, table 29), showed an inferior activity against *Streptococcus plantarum* and a much smaller activity against staphylococci (98).



CXX

In the case of 2,3-diphenyl-5-undecyltetrazolium chloride, the respective figures for other bacteria were:

Bacterium	Minimum Lethal Dose	Minimum Dose for Unhindered Growth
Paratyphus B.	1:19,200	1:102,400
B. Coli	1: 2,400	1: 12,800
Diptheria	1:19,200	1: 38,400
Friedländer B.	1:19,200	1: 25,600

These bacteria were observed to become stained a deep red and the same authors observed that various plant and animal tissues, in particular those of pure yeast and of germinating seeds, were penetrated by the colorless tetrazolium salt solution and stained in a similar manner. The insoluble formazans were clearly being produced in certain centers, especially in the cell nuclei, by reduction, and this was attributed to fermentative processes. Moewus (124) experimented with cress seedlings and showed that immersion in a solution of triphenyltetrazolium chloride or of 2,3-diphenyl-5-undecyltetrazolium chloride caused a red staining of the cotyledons and the growing root-tips.

Lakon (111, 112) at the same time substituted triphenyltetrazolium chloride (T.T.C.) for sodium selenite in his "topographic" method for testing the germinating ability of seeds of oats, rye, wheat, and barley. Only viable seeds were shown to develop a red stain in the embryo. T.T.C. was shown to be much more effective than sodium selenite, and these results were confirmed by numerous other workers. Mattson, Jensen, and Dutcher (120), for example, showed that the same staining could be observed in the fleshy part of apples, oranges, and pears, in the gill areas of mushrooms, in carrot roots, potatoes, young leaves, in the stigma and ovaries of certain pollinated flowers, in bull spermatozoa, and in the blastoderms of hens' eggs. They pointed out that this staining could not be due to sugars, which reduce at pH 11 and not below, since the observed reduction took place at pH 7 or less. Tissues which were heated to 82°C. or higher (which would destroy enzyme activity) lost their ability to reduce tetrazolium salts. The conditions in which T.T.C. is reduced in brewers' yeast and yeast extracts have been studied in detail (78). Hewitt and Agarwala (88) have recently suggested that there is a parallelism between the reducing activity of plant tissues and the distribution of molybdenum in them. (For a more complete bibliography of the use of tetrazolium salts as biological stains, see reference 172.)

This reduction of tetrazolium salts and consequent staining has been shown to be due to enzyme activity, especially that of the dehydrogenase system (coenzyme I and II). Reductions of this kind are achieved at a neutral pH, whereas other constituents of cell fluids, such as glutathione, cysteine, and ascorbic acid, can only reduce tetrazolium salts at a pH > 9. Dehydrogenase-coenzyme I reduces triphenyltetrazolium chloride at pH 6.6. The presence of particular dehydrogenases has been demonstrated by the use of selected tetrazolium salts (89, 160, 165). Thus, T.T.C. and "neotetrazolium salt" (page 431) were reduced in the anaerobic incubation of pleuropneumonia-like organisms and the presence of lactic dehydrogenase and of flavoprotein oxidation was demonstrated (173). Shelton and Schneider (169) have concluded, after examination of the suitability of various tetrazolium salts for use in localizing cell dehydrogenase activity, that the mediation of enzymes other than dehydrogenases may be required in the reduction of tetrazolium salts.

The redox potentials of various tetrazolium salts were measured potentiometrically (103) and polarographically (48a) and the values shown to resemble those of the biological processes of living cells, so that it is possible that these compounds act as electron acceptors for many pyridine-nucleotide-dehydro-

genases. Emphasis has been laid (120) upon the value of such an oxidizing agent, which is unusual in possessing a leuco form stable to atmospheric oxygen and a highly colored reduced form.

Further reduction beyond the formazan stage occurs in the bacterial reduction of 5-methyl-2,3-diphenyltetrazolium chloride (171), so that the red stain due to the formazan becomes gradually paler. The chemical reduction to hydrazidine and amidrazone described on page 412 is apparently paralleled. Other workers (99) have also observed the further reduction of formazans by bacterial and tumor cells.

von Hayer (84) injected triphenyltetrazolium chloride into guinea pigs and observed that after death particular organs were more highly stained than others; the liver and kidneys, seats of particularly high enzyme activity, were the most intensely stained. A tetrazolium salt substituted with a radioactive iodine atom has been prepared (164) to facilitate the study of the distribution of the compound, or of the formazan formed by reduction, in the tissues of mice. Experiments in the staining of nervous tissue with tetrazolium salt solutions have been described (30); the grey brain tissue was stained more intensely than the white.

Tetrazolium compounds have been applied by several workers to the problem of tumor-cell chemistry. Cells such as mouse-ascites tumor are very rapidly and intensely stained by tetrazolium salt solutions, and dehydrogenases of the cell granules have been detected by the observation that reduction occurred only in the presence of *l*-phenylalanine, tyrosine, and glycine (90, 91). In other experiments, using Walker carcinoma, patulin was found to inhibit the reduction, while hypoxanthine, phosphates, and rat serum enhanced it.

Furthermore it has been found that the reduction in many plant and animal tissues is intracellular and occurs in a particular part of the cell (33, 170). In mouse-ascites tumor cells, the reduction region is perinuclear and appears to be associated with the mitochondria present.

Straus, Cheronis, and Straus (174) attempted to develop the tumor-staining ability of tetrazolium salts to provide an early diagnosis of the presence of tumor cells. But subsequent work has shown that, even if these cells do reduce tetrazolium salts more rapidly than normal cells, the difference cannot be used to provide a diagnosis. The reality of a significant difference in the reducing action of normal and tumor cells has been rendered more questionable by experiments with the radioactive tetrazolium salt referred to above (164). Furthermore, tumor cells which have been stained by reduction of tetrazolium salts do not lose their ability to develop tumors when transferred to healthy mice (171). This accords with results obtained by Lettré (114, 115) with other reduction indicators. Experiments on the growth of fibroblasts of dogs' hearts and of embryonic gut tissue showed that the growth was hindered by triphenyltetrazolium chloride at a concentration of one part in 100,000 of solution, although no reduction was observed.

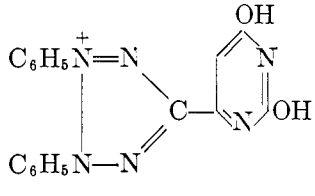
Siegert, Brückel, and Ried (171) investigated the reducing properties of human blood with the help of tetrazolium salts. Such properties were shown to

be attributable to white blood corpuscles, whilst cell-free plasma and erythrocytes were ineffective. One interesting observation made was that tumor carriers and animals infected in other ways yield blood which reduces tetrazolium salts much more effectively than the blood of healthy animals. This again has not provided a tumor diagnostic. The possible advantages of tetrazolium salts in further work on tumor research are discussed by these authors. Hölscher (89) has reported that after treatment with mitotic poisons, the reducing ability of both blood and of tumor cells was very much diminished. He also observed (91) that the light-refracting granules of tumor cells were stained red, but not the cytoplasm and nucleus. This led Seyfarth (167) to the conclusion that mitochondria in normal cells are bodies independent of the normal constituents of cells. This author demonstrated in addition that the chondriosomes of germ cells in *Monilia albicans* are able to reduce tetrazolium salts. The reducing ability of tubercle bacilli has recently been demonstrated (196).

Bielig and Querner (34) used tetrazolium salts as reduction indicators in the growth of marine animals in sea water.

Little work has been reported on the use of tetrazolium salts in other forms of chemotherapy. Triphenyltetrazolium bromide and 2,5-diphenyl-3-*p*-tolyltetrazolium bromide have been shown to be inactive against filariasis (166). A recent report of a number of new tetrazolium salts (2) refers to but does not describe their biological potentialities, but the same workers have subsequently reported (117) a slight activity among tetrazolium salts substituted with amino groups against Influenza A and mouse pneumonitis viruses in mice. Other compounds have been tested against tubercle bacilli.

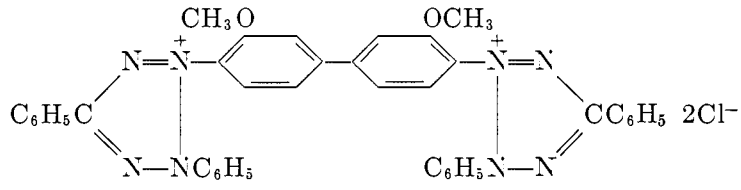
Ludolphy (118) attempted to enhance the ability of tetrazolium salt solutions to penetrate cells by incorporating a suitable pyrimidine nucleus into the molecule. He prepared CXXI but this compound was found to have a high toxicity (0.0002 g. per mouse was lethal) and no special advantages.



CXXI

One major difficulty in much of the biochemical work described above has been that of distinguishing the red formazan stain from the color of blood and other tissues. Much recent work has been aimed at the synthesis of tetrazolium salts which reduce to deep blue formazans, which would provide a clear contrasting stain for microscopic examination. This objective was partly satisfied by the discovery of the so-called "blue tetrazolium" (CXXII) (160), which is

reduced to an intensely blue-black formazan. In solution the formazan is of a purple shade, rather than a true blue.

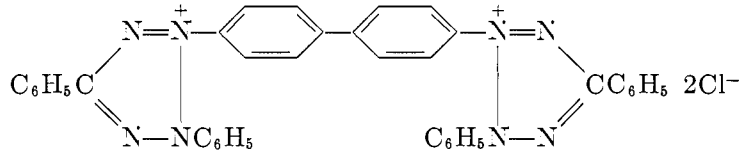


CXXII

An extensive series of ditetrazolium salts, mostly related to CXXII, have been described recently (153, 154) in a further search for pure blue formazans. From a variety of compounds containing various aromatic and heterocyclic groups, several blue types have emerged, the essential structural features being the linkage of two formazan radicals through *o*-dianisidine, and the use of phenylhydrazones of furfural or of positively substituted benzaldehydes. Such ditetrazolium salts have been shown (153) to be reduced by various microorganisms (such as tetanus bacilli, gas gangrene bacilli, or *Bacterium subtilis*) with intense staining.

The same workers (154, 156) showed that mono- and diformazans containing various heterocyclic groups, including some with three substituent heterocyclic nuclei, were red in color and not of value for staining in this context. Diformazans from nitro- or halogen-substituted benzaldehydes, from heterocyclic hydrazones, and from benzidine and *p*-phenylenediamine were also red in color.

Another ditetrazolium salt whose staining potentialities have been examined in detail is "Neo-tetrazolium salt" (CXXIII, table 34) (1, 203):



CXXIII

Antopol and his coworkers have applied this compound in solution to a variety of tissues, tumor cells, and microörganisms. Another compound, 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride, is recommended because it has a much lower photosensitivity than triphenyltetrazolium chloride and stains very rapidly with a minimum of diffusion into non-staining tissues (3). CXXIII and several other ditetrazolium salts have been reported to possess a curare-like action in mice (163a).

Some other ditetrazolium salts, and monotetrazolium salts bearing diphenylyl groups, were prepared by Jerchel and Fischer (100), partly with the aim of improving the staining properties and also to examine the effect on solubility and toxicity. T.T.C. is readily soluble in water (two crystalline forms reported

have solubilities of 25 g. and 16 g. in 100 g. of water at 25°C.) (70), but substitution causes a marked reduction in solubility. Since they contain two quaternary centers, the ditetrazolium salts were thought likely to be more soluble, and this was confirmed. The diphenylyl-substituted salts were relatively insoluble. The qualified success of attempts to increase the solubility by changing the anion, notably by converting chlorides to isethionate by reaction in aqueous-alcoholic solution with silver isethionate (117), has prompted attempts to introduce specifically solubilizing groups into the tetrazolium molecule. A series of these salts carrying trimethylammonium groups on aromatic rings has been prepared (see page 384), and in spite of various substitutions elsewhere in the molecule, highly water-soluble salts have been obtained (126). The corresponding formazans are also appreciably water-soluble (0.1–3 per cent).

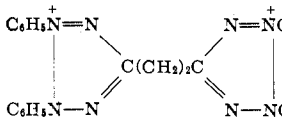
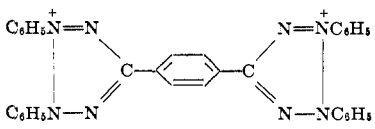
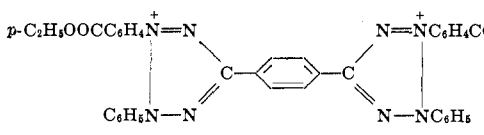
TABLE 5

Toxicity of triphenyltetrazolium chloride

Milligrams of T.T.C. per Gram of Mouse	Proportion of Deaths	Time Interval
		hr.
(a) Intraperitoneal:		
0.005-0.01	1/8	90
0.011-0.02	5/11	5-41
0.021-0.03	9/9	1-6
0.031-0.07	8/8	¼-1
(b) Intravenous:		
0.0005-0.005	0/3	
0.005-0.02	3/3	2-14

TABLE 6

*Comparison of toxicities of tetrazolium salts towards fish**

Tetrazolium Salt	Concentration Used		
	0.1	0.05	0.01
5-Methyl-2,3-diphenyl-	4	4	8
2,3,5-Triphenyl- (T.T.C.)	1	2	5
5-Diphenylyl-2,3-diphenyl-	2	2	2
2-Diphenylyl-3,5-diphenyl-		All six died	
2,3-Bisdiphenylyl-5-phenyl-		4	5
	9	9	10
	1	5	7
	2	7	9

* The figures opposite the compounds denote the number of days during which four out of six fish had died.

B. TOXICITY OF TETRAZOLIUM SALTS

Another important problem created by the biological use of tetrazolium salts is their toxicity. The toxicity of T.T.C. has been measured (100) with the results shown in table 5.

Comparisons of the toxicities of several tetrazolium salts were made by the same workers on the fish *Barbus conchonius* (see table 6).

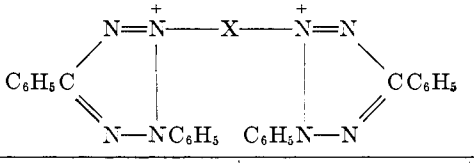
All these compounds, except the one derived from 4-aminobiphenyl, showed a lower toxicity than T.T.C. itself. Lettré and Albrecht (116) have tested the toxicity of T.T.C. on the fibroblasts of dogs' hearts.

Other measurements of toxicity recently reported (163a) are shown in table 7 as minimum lethal doses in mice.

Rutenberg, Gofstein, and Seligman (160) found that the ratios of toxicities of "blue tetrazolium," T.T.C., and 2-*p*-iodophenyl-3,5-diphenyltetrazolium chloride were 22:4:1 intravenously and 10:2:1 intraperitoneally. Early work (140) described the effect of 5-carbethoxy-2,3-diphenyltetrazolium chloride by subcutaneous injection in rabbits, the successive symptoms being sickness, paralysis of the heart and restriction of circulation, and death; subcutaneous injection of 2-*p*-hydroxyphenyl-3,5-diphenyltetrazolium chloride into rabbits

TABLE 7

Toxicities of tetrazolium salts of the structure



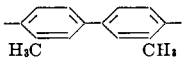
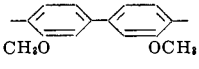
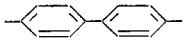
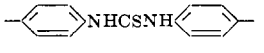
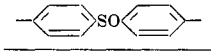
X	Anion	Lethal Dose
		g./kg. of body weight
	I	0.002
	I	0.0015
	Cl	0.005
	I	0.014
	I	0.0035

TABLE 8

Effect of sugar molecules in tetrazolium salts on toxicity

	Dose (g./kg. of Body Weight)		
	T.T.C.	*d*-Galactodiphenyl-tetrazolium chloride	Pentaacetyl-*d*-galactodiphenyl-tetrazolium chloride
Rats killed at once	0.028	0.10	0.066
Rats symptom-free	0.002	0.075	0.055

and frogs caused paralysis of the extremities in small doses but 0.16 g./kg. was fatal (180). Hungarian workers (205) have recently shown that the toxicity of tetrazolium salts can be reduced by the introduction of sugar groups attached to carbon (page 406). These compounds do not appear very easy to synthesize. Comparisons were made between these tetrazolium salts and T.T.C. in respect of their toxicities to rats.

No information has been published about the toxicity of formazans, but Jerchel and Kuhn (102) showed that ω-phenylbenzamidrazone, obtained by the reduction of triphenylformazan with sodium dithionite (page 413), is one-tenth as toxic as T.T.C. when injected intraperitoneally into mice.

C. OTHER USES

Finally, three non-biological applications of tetrazolium salts should be mentioned. Weiner (192) has reported the use of T.T.C. as an indicator for reducing substances in alkaline solution; Mattson and Jensen (119) have described its use in the colorimetric determination of reducing sugars at 4900 Å.; Wallenfels (178) has developed a quantitative test for reducing sugars using solutions of tetrazolium salts in paper chromatography.

It is hoped that this brief survey has given some indication of the wide interest today in the biological properties of tetrazolium compounds. A very considerable proportion of this work has centered on the well-known triphenyltetrazolium salts, and many aspects of the general chemistry of these compounds and of formazans remain to be investigated. Much is still obscure in the synthesis of formazans; methods available are beset by limitations for which theoretical interpretation is sadly lacking. Although very many examples of the condensation of diazonium salts with phenylhydrazone have been reported, little systematic work, aimed at an elucidation of the controlling factors, has been attempted. One particularly intriguing puzzle, which seems to have been neglected, is that of the comparative colors of formazans and tetrazolium salts. Since, in the modern view, these compounds both possess mesomeric structures, differing only in the presence of a hydrogen bridge in the one and of a positive charge in the other, their light-absorbing properties might be expected to be similar. The great variation in these properties, leading to intensely red or violet formazans and to colorless tetrazolium salts, still requires explanation.

XV. Tables of Formazans

Tables 9–26 list all of the formazans which it is believed have been described in the literature to the end of 1953. In these tables the method of synthesis is indicated according to the lettering used in Section VI, A–G.

XVI. Tables of Tetrazolium Salts

Tables 27–35 list the tetrazolium salts which have been described in the literature to the end of 1953. Some compounds reported on in 1954 or not yet described in the literature are included. In these tables the methods of synthesis are designated by the letters A to H, which have the significance indicated below:

A = oxidation of formazan with isoamyl nitrite
B = oxidation of formazan with nitrous fumes

C = oxidation of formazan with lead tetraacetate
D = oxidation of formazan with mercuric oxide
E = oxidation of formazan with butyl nitrite
F = modification of preëxistent tetrazolium salts
G = oxidation of formazans with halogenoimides
H = aerial oxidation

XVII. References

(1) Antopol, W., Glaubach, S., and Goldman, L.: Public Health Repts. (U.S.) **63**, 1231 (1948).
(2) Ashley, J. N., Davis, B. M., Nineham, A. W., and Slack, R.: J. Chem. Soc. **1953**, 3881.
(3) Atkinson, E. H., Melvin, S., and Fox, S. W.: Science **111**, 385 (1950).
(4) Backer, H. J.: Rec. trav. chim. **70**, 733 (1951).
(5) Bamberger, E.: Ber. **24**, 3260 (1891).
(6) Bamberger, E.: Ber. **25**, 3547 (1892).
(7) Bamberger, E.: Ber. **27**, 155 (1894).
(8) Bamberger, E.: Arch. sci. phys. et nat. [iv] **6**, 384 (1898); Chem. Zentr. **1898**, II, 1050.
(9) Bamberger, E.: Ber. **44**, 3743 (1911).
(10) Bamberger, E., and Billeter, O.: Vierteljahrsschr. naturforsch. Ges. Zürich **48**, 329 (1903).
(11) Bamberger, E., and Billeter, O.: Helv. Chim. Acta **14**, 219 (1931).
(12) Bamberger, E., and Grob, J.: Ber. **34**, 523 (1901).
(13) Bamberger, E., and Gruyter, P. de: Ber. **26**, 2385 (1893).
(14) Bamberger, E., and Gruyter, P. de: Ber. **26**, 2783 (1893).
(15) Bamberger, E., and Gruyter, P. de: J. prakt. Chem. [2] **64**, 222 (1901).
(16) Bamberger, E., and Kuhlemann, F.: Ber. **26**, 2978 (1893).
(17) Bamberger, E., and Lorenzen, J.: Ber. **25**, 3539 (1892).
(18) Bamberger, E., and Müller, J.: Ber. **27**, 147 (1894).
(19) Bamberger, E., and Müller, J.: J. prakt. Chem. [2] **64**, 199 (1901).
(20) Bamberger, E., Padova, R., and Ormerod, E.: Ann. **446**, 260 (1925).
(21) Bamberger, E., and Pemsel, W.: Ber. **36**, 53, 85 (1903).
(22) Bamberger, E., and Schmidt, O.: Ber. **34**, 574 (1901).
(23) Bamberger, E., and Schmidt, O.: Ber. **34**, 2001 (1901).
(24) Bamberger, E., Schmidt, O., and Levinstein, H.: Ber. **33**, 2043, 2050 (1900).
(25) Bamberger, E., and Wheelwright, E. W.: Ber. **25**, 3201 (1892).
(26) Bamberger, E., and Wheelwright, E. W.: J. prakt. Chem. [2] **65**, 123 (1902).
(27) Bamberger, E., and Witter, H.: Ber. **26**, 2786 (1893).
(28) Bamberger, E., and Witter, H.: J. prakt. Chem. [2] **65**, 139 (1902).
(29) Bamberger, E. and Wulz, P.: Ber. **24**, 2793 (1891).
(30) Becker, H., and Quadbeck, G.: Naturwissenschaften **37**, 565 (1950).
(31) Benson, F. R.: Chem. Revs. **41**, 1 (1947).
(32) Benson, F. R., Hartzel, L. W., and Savell, W. L.: J. Am. Chem. Soc. **73**, 4457 (1951).
(32a) Benson, F. R., Otten, E. A., and Schachat, R. E.: J. Am. Chem. Soc. **76**, 1695 (1954).
(33) Bielig, H. J., Kausche, G. A., and Haardick, H.: Z. Naturforsch. **5b**, 80 (1950).
(34) Bielig, H. J., and Querner, H.: Z. Naturforsch. **4b**, 21 (1949).
(35) Billmann, J. H., and Cleland, E. S.: Org. Syntheses **25**, 38 (1945).
(36) Bodforss, S.: Ber. **59**, 670 (1926).
(36a) Borsche, W., and Manteuffel, R.: Ann. **505**, 189 (1933).
(37) Borsche, W., and Manteuffel, R.: Ann. **512**, 97 (1934).
(38) Breusch, F. L., and Keskin, H.: Rev. faculté sci. univ. Istanbul **9A**(i), 30 (1944).

(39) Busch, M., Achterfeldt, F., and Seufert, R.: J. prakt. Chem. [2] **92,** 1 (1915).
(40) Busch, M., and Beust, R. von.: Ber. **58,** 442 (1925).
(41) Busch, M., and Frey, R.: Ber. **36,** 1362 (1903).
(42) Busch, M., and Kunder, H.: Ber. **49,** 2345 (1916).
(43) Busch, M., and Pfeiffer, H.: Ber. **59,** 1162 (1926).
(44) Busch, M., and Schmidt, K.: J. prakt. Chem. **129,** 151 (1931).
(45) Busch, M., and Schmidt, R.: Ber. **63,** 1950 (1930).
(46) Busch, M., and Schmidt, R.: J. prakt. Chem. [2] **131,** 182 (1931).
(47) Busch, M., and Schmidt, W.: Ber. **62,** 1454 (1929).
(48) Busch, M., and Wolbring, W.: J. prakt. Chem. [2] **71,** 366 (1905).
(48a) Campbell, H., and Kane, P. O.: Private communication.
(49) Canonica, L.: Gazz. chim. ital. **79,** 738 (1949).
(50) Capka, O.: Chem. Zvesti **2,** 1 (1948); Chem. Abstracts **44,** 1523 (1950).
(51) Cazeneuve, P.: Compt. rend. **130,** 1478 (1900).
(52) Cazeneuve, P.: Bull. soc. chim. France [3] **23,** 593 (1900).
(53) Cazeneuve, P.: Bull. soc. chim. France [3] **25,** 375 (1901).
(54) Chattaway, F. D., Drewitt, J. G. N., and Parkes, G. D.: J. Chem. Soc. **1936,** 1693.
(55) Chattaway, F. D., and Walker, A. J.: J. Chem. Soc. **127,** 975 (1925).
(56) Ciba Ltd.: Swiss patent 246,475; Chem. Abstracts **43,** 5198 (1949).
(57) Claisen, L.: Ber. **25,** 747 (1892).
(58) Claisen, L.: Ann. **287,** 360 (1895).
(59) Demuth, R., and Meyer, V.: Ann. **256,** 35 (1890).
(60) Duffin, G. F., and Kendall, J. D.: J. Chem. Soc. **1954,** 408.
(60a) Duffin, G. F., and Kendall, J. D.: Private communication.
(61) Eibner, A., and Hofmann, K. A.: Ber. **37,** 3071 (1904).
(62) Euler, H. von, and Hasselquist, H.: Arkiv Kemi **3,** 139 (1951).
(63) Feigl, F., and Lederer, F. L.: Monatsh. **45,** 63 (1924).
(64) Fichter, F. R., and Fröhlich, J.: Chem. Zentr. **1903, II,** 426.
(65) Fichter, F. R., and Philipp, K.: J. prakt. Chem. [2] **74,** 315 (1906).
(66) Fichter, F. R., and Schiess, E.: Ber. **33,** 752 (1900).
(67) Fischer, E.: Ann. **190,** 120 (1878).
(68) Fischer, E.: Ber. **22,** 1930 (1889).
(69) Fischer, E., and Besthorn, K.: Ann. **212,** 316 (1882).
(70) Fox, S. W., and Atkinson, E. H.: J. Am. Chem. Soc. **72,** 3629 (1950).
(71) Freund, M.: Ber. **24,** 4178 (1891).
(72) Friese, P.: Ber. **8,** 1078 (1875).
(73) Frobenius, W.: German patent 423,321; Chem. Zentr. **1926, I,** 2422.
(74) Fusco, R., and Romani, R.: Gazz. chim. ital. **76,** 419 (1946).
(75) Fusco, R., and Romani, R.: Gazz. chim. ital. **78,** 332 (1948).
(76) Grammaticakis, P.: Compt. rend. **225,** 684 (1947).
(77) Grammaticakis, P.: Compt. rend. **234,** 528 (1952).
(78) Gunz, F. W.: Nature **163,** 98 (1949).
(79) Hadaçek, J., and Vendraçek, M.: Chem. Obzor. **22,** 197 (1947).
(79a) Hauptmann, H., and Périsse, A. C. de M.: Experientia **10,** 60 (1954).
(80) Hausser, K. H.: Naturwissenschaften **36,** 313 (1949).
(81) Hausser, I., Jerchel, D., and Kuhn, R.: Chem. Ber. **82,** 195 (1949).
(82) Hausser, I., Jerchel, D., and Kuhn, R.: Chem. Ber. **82,** 515 (1949).
(83) Hausser, I., Jerchel, D., and Kuhn, R.: Chem. Ber. **84,** 651 (1951).
(84) Hayer, H. von: Naturwissenschaften **37,** 262 (1950).
(85) Heller, G.: Ann. **263,** 269 (1891).
(86) Henrich, F., Reichenburg, W., Nachtigall, G., Thomas, W., and Baum, C.: Ann. **376,** 125 (1910).
(87) Henrich, F., and Thomas, W.: Ber. **40,** 4924 (1907).
(88) Hewitt, E. J., and Agarwala, S. C.: Nature **169,** 545 (1952).

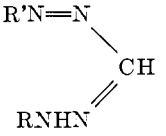
(89) HÖLSCHER, H. A.: Z. Krebsforsch. **56,** 587 (1950).
(90) HÖLSCHER, H. A.: Naturwissenschaften **38,** 116 (1951).
(91) HÖLSCHER, H. A.: Z. Krebsforsch. **57,** 353, 634 (1951).
(92) HUBBARD, D. M., AND SCOTT, E. W.: J. Am. Chem. Soc. **65,** 2390 (1943).
(93) HÜNIG, S., AND BOES, O.: Ann. **579,** 28 (1953).
(94) HUNTER, L., AND ROBERTS, C. B.: J. Chem. Soc. **1941,** 820, 822.
(95) IRVING, H., AND BELL, C. F.: Nature **169,** 756 (1952).
(96) IRVING, H., AND BELL, C. F.: J. Chem. Soc. **1953,** 3538.
(97) JAGERSPACHER, C.: Ber. **28,** 1283 (1895).
(98) JERCHEL, D.: Ber. **75,** 75 (1942).
(99) JERCHEL, D.: FIAT Review of Biochemistry **1947,** I, 59.
(100) JERCHEL, D., AND FISCHER, H.: Ann. **563,** 200, 208 (1949).
(101) JERCHEL, D., AND FISCHER, H.: Ann. **574,** 85 (1951).
(102) JERCHEL, D., AND KUHN, R.: Ann. **568,** 185 (1950).
(103) JERCHEL, D., AND MÖHLE, W.: Ber. **77,** 591 (1944).
(104) JONES, E. C. S., AND KENNER, J.: J. Chem. Soc. **1930,** 919.
(105) KRUMHOLZ, P., AND KRUMHOLZ, E.: Monatsh. **70,** 431 (1937).
(105a) KRUMHOLZ, P., and WATZEK, H.: Monatsh. **70,** 437 (1937).
(106) KUHN, R., AND JERCHEL, D.: Ber. **74,** 941, 949 (1941).
(107) KUHN, R., AND JERCHEL, D.: Ann. **578,** 1 (1952).
(108) KUHN, R., AND MUNZING, W.: Chem. Ber. **86,** 858 (1953).
(109) KUHN, R., AND WEITZ, H. M.: Chem. Ber. **86,** 1199 (1953).
(110) KUL'BERG, L. M., AND LEDNEVA, A. M.: Zhur. Anal. Khim. **2,** 131 (1947); Chem. Abstracts **43,** 5696f (1949).
(111) LAKON, G.: Ber. deut. botan. Ges. **57,** 191 (1939).
(112) LAKON, G.: Ber. deut. botan. Ges. **60,** 299, 434 (1942).
(113) LAPWORTH, A.: J. Chem. Soc. **83,** 1125 (1903).
(114) LETTRÉ, H.: Z. Krebsforsch. **56,** 297 (1949).
(115) LETTRÉ, H.: Angew. Chem. **63,** 421 (1951).
(116) LETTRÉ, H., AND ALBRECHT, M.: Z. physiol. Chem. **279,** 206 (1943).
(117) LIBMAN, D. D., NINEHAM, A. W., AND SLACK, R.: J. Chem. Soc. **1954,** 1565.
(117a) LIN, C.-H., LIEBER, E., AND HORWITZ, J. P.: J. Am. Chem. Soc. **76,** 427 (1954).
(118) LUDOLPHY, E.: Chem. Ber. **84,** 385 (1951).
(119) MATTSON, A. M., AND JENSEN, C. O.: Anal. Chem. **22,** 182 (1950).
(120) MATTSON, A. M., JENSEN, C. O., AND DUTCHER, R. A.: Science **106,** 294 (1947).
(121) MATTSON, A. M., JENSEN, C. O., AND DUTCHER, R. A.: J. Am. Chem. Soc. **70,** 1284 (1948).
(122) MICHAEL, A.: Ber. **38,** 2101 (1905).
(123) MITCHELL, A. D.: *British Chemical Nomenclature.* E. Arnold & Co., London (1948).
(124) MOEWUS, F.: Biol. Zentr. **60,** 143 (1940).
(125) MOSSINI, A.: Ann. chim. farm. **3,** 24 (1940); Chem. Abstracts **34,** 7916 (1940).
(126) NINEHAM, A. W.: To be published.
(126a) NINEHAM, A. W.: Unpublished results.
(127) NINEHAM, A. W., PAIN, D. L., AND SLACK, R.: J. Chem. Soc. **1954,** 1568.
(128) Nomenclature Report, Chem. Abstracts **39,** 5964 (1945).
(129) OESPER, R. E., AND KLINGENBERG, J. J.: J. Org. Chem. **13,** 309 (1948).
(129a) PAIN, D. L.: Private communication.
(130) PAIN, D. L., COTTRELL, H. J., AND SLACK, R.: J. Chem. Soc. **1954,** 2968.
(131) PARKES, G. D., AND ALDIS, B. C.: J. Chem. Soc. **1938,** 1841.
(132) PECHMANN, H. VON: Ber. **25,** 3175 (1892).
(133) PECHMANN, H. VON: Ber. **27,** 320 (1894).
(134) PECHMANN, H. VON: Ber. **27,** 1679 (1894).
(135) PECHMANN, H. VON: Ber. **28,** 876 (1895).
(136) PECHMANN, H. VON: Ber. **29,** 2166 (1896).
(137) PECHMANN, H. VON, AND JENISCH, C.: Ber. **24,** 3255 (1891).

(138) PECHMANN, H. VON, AND RUNGE, P.: Ber. **27,** 323 (1894).
(139) PECHMANN, H. VON, AND RUNGE, P.: Ber. **27,** 1693 (1894).
(140) PECHMANN, H. VON, AND RUNGE, P.: Ber. **27,** 2920 (1894).
(141) PECHMANN, H. VON, AND WEDEKIND, E.: Ber. **28,** 1688 (1895).
(142) PINNER, A.: Ber. **17,** 183 (1884).
(143) PONZIO, G., AND GASTALDI, C.: Gazz. chim. ital. **44,** 257 (1914).
(144) PRAGER, B.: Ann. **338,** 360 (1905).
(145) QUILICO, A., AND FRERI, M.: Gazz. chim. ital. **60,** 606 (1930).
(146) RABISCHONG, J.: Bull. soc. chim. France [3] **31,** 82 (1904).
(147) RAGNO, M., AND BELLOMO, A.: Gazz. chim. ital. **78,** 45 (1948).
(148) RAGNO, M., AND BRUNO, S.: Gazz. chim. ital. **76,** 485 (1946).
(149) RAGNO, M., AND BRUNO, S.: Gazz. chim. ital. **77,** 12 (1947).
(150) RAGNO, M., AND ORESTE, D.: Gazz. chim. ital. **78,** 228 (1948).
(151) RAPOPORT, H., AND BONNER, R. M.: J. Am. Chem. Soc. **72,** 2783 (1950).
(152) RIED, W.: Angew. Chem. **64,** 391 (1952).
(153) RIED, W., AND GICK, H.: Ann. **581,** 16 (1953).
(154) RIED, W., GICK, H., AND OERTEL, G.: Ann. **581,** 29 (1953).
(155) RIED, W., AND HILLENBRAND, H.: Ann. **581,** 44 (1953).
(156) RIED, W., AND HOFFSCHMIDT, R.: Ann. **581,** 23 (1953).
(157) RIED, W., AND WILK, M.: Ann. **581,** 49 (1953).
(158) ROTHENBURG, R. VON: Ber. **27,** 689 (1894).
(159) RUPE, H., AND BURCKHARDT, E.: Ber. **49,** 2547 (1916).
(160) RUTENBERG, A. M., GOFSTEIN, R., AND SELIGMAN, A. M.: Cancer Research **10,** 113 (1950).
(161) SCOTT, F. L., O'SULLIVAN, D. A., AND REILLY, J.: J. Chem. Soc. **1951,** 3508.
(162) SCOTT, F. L., O'SULLIVAN, D. A., AND REILLY, J.: Chemistry & Industry **1952,** 782.
(163) SCOTT, F. L., O'SULLIVAN, D. A., AND REILLY, J.: J. Am. Chem. Soc. **75,** 5309 (1953).
(163a) SEILER, H., AND SCHMID, H.: Helv. Chim. Acta **37,** 1 (1954).
(164) SELIGMAN, A. M., GOFSTEIN, R., AND RUTENBERG, A. M.: Cancer Research **9,** 366 (1949).
(165) SELIGMAN, A. M., AND RUTENBERG, A. M.: Science **113,** 317 (1951).
(166) SEWELL, P., AND HAWKING, F.: Brit. J. Pharmacol. **5,** 239 (1950).
(167) SEYFARTH, W.: Naturwissenschaften **39,** 91 (1952).
(168) SEYHAN, M.: Rev. faculté sci. univ. Istanbul **17A,** 182 (1952); Chem. Abstracts **47,** 12390 (1953).
(168a) SEYHAN, M.: Rev. faculté sci. univ. Istanbul **17A,** 299 (1952); Chem. Abstracts **48,** 3348 (1954).
(169) SHELTON, E., AND SCHNEIDER, W. C.: Anat. Record **112,** 61 (1952).
(170) SIEGERT, R.: Angew. Chem. **61,** 258 (1949).
(171) SIEGERT, R., BRÜCKEL, K. W., AND RIED, W.: Z. ges. exptl. Med. **117,** 626 (1951).
(172) SMITH, F. E.: Science **113,** 753 (1951).
(173) SOMERSON, N. L., AND MORTON, H. E.: J. Bact. **65,** 245 (1953).
(174) STRAUS, F. H., CHERONIS, N. D., AND STRAUS, E.: Science **108,** 113 (1948).
(175) TRÖGER, J., AND BERNDT, A.: J. prakt. Chem. [2] **102,** 1 (1921).
(176) VOSWINCKEL, H.: Ber. **36,** 2484 (1903).
(177) WALKER, T. K.: J. Chem. Soc. **123,** 2775 (1923).
(178) WALLENFELS, K.: Naturwissenschaften **37,** 491 (1950).
(179) WALTHER, R. VON: J. prakt. chem. [2] **53,** 475 (1896).
(180) WEDEKIND, E.: Ber. **29,** 1846 (1896).
(181) WEDEKIND, E.: Ber. **30,** 444 (1897).
(182) WEDEKIND, E.: Ber. **30,** 2993 (1897).
(183) WEDEKIND, E.: Ber. **31,** 473 (1898).
(184) WEDEKIND, E.: Ber. **31,** 479 (1898).
(185) WEDEKIND, E.: Ber. **31,** 2353 (1898).

(186) WEDEKIND, E.: Ann. **300,** 239 (1898).
(187) WEDEKIND, E.: Ber. **32,** 1919 (1899).
(188) WEDEKIND, E.: Ann. **442,** 119 (1925).
(189) WEDEKIND, E., AND BRONSTEIN, S.: Ann. **307,** 293 (1899);
(190) WEDEKIND, E., AND NISSEN, P.: Ann. **295,** 324 (1897).
(191) WEDEKIND, E., AND STAUWE, L.: Ber. **31,** 1746 (1898).
(192) WEINER, S.: Chemist-Analyst **37,** 55 (1948).
(193) WEISSBACH, H.: J. prakt. Chem. [2] **67,** 395 (1903).
(194) WEYGAND, F., AND FRANK, I.: Z. Naturforsch. **3b,** 377 (1948).
(195) WHITELEY, M. A., AND YAPP, D.: J. Chem. Soc. **1927,** 521.
(196) WILLIS, H. S., VANDIVIERE, H. M., AND GENTRY, W. H.: Am. J. Med. Sci. **225,** 410 (1953).
(197) WISLICENUS, W.: Ber. **25,** 3456 (1892).
(198) WISLICENUS, W., AND JENSEN, A.: Ber. **25,** 3448 (1892).
(199) WIZINGER, R., AND BIRO, V.: Helv. Chim. Acta **32,** 901 (1949).
(200) WIZINGER, R., AND HERZOG, H.: Helv. Chim. Acta **34,** 1202 (1951).
(201) WIZINGER, R., AND HERZOG, H.: Helv. Chim. Acta **36,** 531 (1953).
(202) WOHL, A., AND SCHIFF, H.: Ber. **33,** 2741 (1900).
(203) WOOD, R. M.: Science **112,** 86 (1950).
(204) ZEMPLÉN, G., AND MESTER, L.: Acta Chim. Acad. Sci. Hung. **2,** 9 (1952); Chem. Abstracts **48,** 1966 (1954).
(205) ZEMPLÉN, G., MESTER, L., AND ECKHART, E.: Chem. Ber. **86,** 472 (1953).
(206) ZEMPLÉN, G., MESTER, L., AND MESSMER, A.: Chem. Ber. **86,** 697 (1953).
(207) ZEMPLÉN, G., MESTER, L., MESSMER, A., AND ECKHART, E.: Acta Chim. Acad. Sci. Hung. **2,** 25 (1952); Chem. Abstracts **48,** 1966 (1954).

TABLE 9

Formazans unsubstituted at position 3

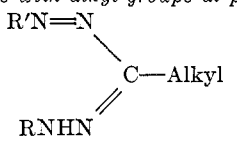


R	R'	Method of Synthesis	Yield	Melting Point	Appearance	References
			per cent	°C.		
C_6H_5	C_6H_5	A1, A2, B, D	19, 46	119-120	Red needles, violet reflex	(25, 26, 39, 40, 58, 82, 132, 140, 143, 179)*†
C_6H_5	4-$CH_3C_6H_4$	D		116-117	Ruby-red leaflets	(139)
4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	A2		105 d.	Brownish red needles, metallic reflex	(41)
2,4-$(CH_3)_2C_6H_3$	C_6H_5	A1		162-163	Brownish red needles, green reflex	(40)
4-$CH_3OC_6H_4$	4-$CH_3OC_6H_4$	A2		88	Red prisms	(141)
4-BrC_6H_4	4-BrC_6H_4	A2, B		114-115 177-178	Dark red prisms	(48, 96, 143)
C_6H_5	2,4-$Br_2C_6H_3$	A1		198-200	Orange-red	(96)
2-IC_6H_4	2-IC_6H_4	A2		168-169 d.	Brownish red needles, metallic reflex	(48)
2-$O_2NC_6H_4$	2-$O_2NC_6H_4$	A2	5	186-187	Dark red needles	(48)
3-$O_2NC_6H_4$	3-$O_2NC_6H_4$	A2			Brownish red needles	(48, 141)
4-$O_2NC_6H_4$	4-$O_2NC_6H_4$	A2			Brownish red needles	(48, 141)

* The corresponding tetrazolium salt has been described.
† Acetyl derivative, m.p. 188-188.5°C.

TABLE 10

Formazans with alkyl groups at position 3



R	R'	Alkyl	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
C_6H_5	C_6H_5	CH_3	A1 A2 B E G	44–94	125	Bright orange needles, blue reflex	(10, 11, 18, 19, 21, 82, 106, 148, 176, 177, 182)*
C_6H_5	2-$O_2NC_6H_4$	CH_3	A1		138	Orange-red needles, gold reflex	(148)
C_6H_5	3-$O_2NC_6H_4$	CH_3	A1		165	Yellow powder	(148)
C_6H_5	4-$O_2NC_6H_4$	CH_3	A1		154	Rust-red needles, bronze reflex	(11, 148)
2-$O_2NC_6H_4$	2-$O_2NC_6H_4$	CH_3	A1	"Very small"	165	Violet-red, metallic reflex	(148)
4-$O_2NC_6H_4$	2-$O_2NC_6H_4$	CH_3	A1		160	Brownish red, metallic reflex	(148)
4-$O_2NC_6H_4$	3-$O_2NC_6H_4$	CH_3	A1		204-205	Brick-red needles	(148)
4-$O_2NC_6H_4$	4-$O_2NC_6H_4$	CH_3	A1	91	229-230	Red rhombs, metallic reflex	(145, 147, 148)
2,4-$(NO_2)_2C_6H_3$	C_6H_5	CH_3	A1		200–202	Red scales, blue reflex	(149, 150)
2,4-$(NO_2)_2C_6H_3$	2-$O_2NC_6H_4$	CH_3	A1		193	Ruby-red scales, blue reflex	(150)
2,4-$(NO_2)_2C_6H_3$	3-$O_2NC_6H_4$	CH_3	A1		204-205	Orange-red needle, aggregates	(150)
2,4-$(NO_2)_2C_6H_3$	4-$O_2NC_6H_4$	CH_3	A1		200–201	(a) Orange-red, gold reflex (b) Blood-red, blue reflex	(150)
C_6H_5	4-HOC_6H_4	CH_3	A1		148-149		(103)*
C_6H_5	2-$HOOCC_6H_4$	CH_3	A1		200	Deep red needles	(103)*
C_6H_5	4-$HOOCC_6H_4$	CH_3	A1	100	185	Red needles	(103)*
C_6H_5	4-$H_2NSO_2C_6H_4$	CH_3	A1	65	235	Dark red rods	(98)*
C_6H_5	C_6H_5	C_2H_5	A1, A2	63-100	(a) 103 (b) 75	(a) Orange needles (b) Carmine needles	(38, 82, 177)*
C_6H_5	4-$H_2NSO_2C_6H_4$	n-C_3H_7	A1	63	200	Red needles	(98)*
C_6H_5	C_6H_5	iso-C_3H_7	A1		87	Red	(38)*
C_6H_5	C_6H_5	iso-C_4H_9	A1		60	Red	(38)*
C_6H_5	C_6H_5	n-C_5H_{11}	A1		92	Red	(38)*
C_6H_5	C_6H_5	n-C_6H_{13}	A1	81	76	Deep red	(106)*
C_6H_5	4-$H_2NSO_2C_6H_4$	n-C_6H_{13}	A1	100	181	Deep red	(98)*
C_6H_5	C_6H_5	n-C_7H_{15}	A1	47	69	Red needles	(38, 82)
C_6H_5	2-$HOOCC_6H_4$	n-C_7H_{15}	A1	54†	162	Bright red needles	(103)*
C_6H_5	4-$H_2NSO_2C_6H_4$	n-C_7H_{15}	A1	94	176		(98)*
C_6H_5	C_6H_5	n-C_8H_{17}	A1		51	Red	(38)
C_6H_5	C_6H_5	n-C_9H_{19}	A1		40	Red	(38)
C_6H_5	C_6H_5	n-$C_{11}H_{23}$	A1	76	61	Deep red needles	(106)*
C_6H_5	4-BrC_6H_4	n-$C_{11}H_{23}$	A1	82	53	Dark red needles	(106)*
C_6H_5	4-$O_2NC_6H_4$	n-$C_{11}H_{23}$	A1	82	108-109	Red platelets	(106)
C_6H_5	4-$H_2NSO_2C_6H_4$	n-$C_{11}H_{23}$	A1	100	167	Small red needles	(98)*

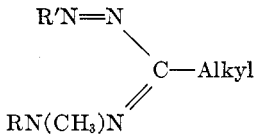
TABLE 10—*Concluded*

R	R'	Alkyl	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
C_6H_5	α-$C_{10}H_7$	*n*-$C_{11}H_{23}$	A1	64†	60	Shining red	(106)*
4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	$-CH_2CH{=}CH_2$	A2	50	117	Canary-yellow prisms	(177)
C_6H_5	C_6H_5	$-C(=CH_2)CH_3$	A1	72	109	Deep red needles	(60)
C_6H_5	C_6H_5	$C_6H_5CH_2-$	A2,B	50	(a) 93.5 d.	Deep orange plates	(176, 177)
					(b) 127	Red leaflets, metallic reflex	(193)
2-$CH_3C_6H_4$	2-$CH_3C_6H_4$	$-CH_2CH_2OH$	A2	34	145	Orange-red needles	(60a)
2-ClC_6H_4	2-ClC_6H_4	$-CH_2CH_2OH$	A2	38-40	147	Red needles, green reflex	(60a)
2-BrC_6H_4	2-BrC_6H_4	$-CH_2CH_2OH$	A2	10.5	155-157	Red needles, green reflex	(60a)
2-$O_2NC_6H_4$	2-$O_2NC_6H_4$	$-CH_2CH_2OH$	A2	4	162	Red needles	(60a)
2-$CH_3OC_6H_4$	2-$CH_3OC_6H_4$	$-CH_2CH_2OH$	A2	12	132-133	Red needles, blue reflex	(60a)
2-$CH_3C_6H_4$	2-ClC_6H_4	$-CH_2CH_2OH$	A1	7, 23	140	Dark red needles, green reflex	(60a)
C_6H_5	C_6H_5	$-CH_2CHOHCH_3$	A2	4	108	Dark red needles, blue reflex	(60a)
2-ClC_6H_4	2-ClC_6H_4	$-CH_2CHOHCH_3$	A2	15-17	124	Orange-red needles	(60a)
2-$CH_3C_6H_4$	2-$CH_3C_6H_4$	$-(CH_2)_2OCOCH_3$	D		118	Orange plates	(60a)
2-ClC_6H_4	2-ClC_6H_4	$-(CH_2)_2OCOCH_3$	D	30	120	Dark red needles	(60a)
2-BrC_6H_4	2-BrC_6H_4	$-(CH_2)_2OCOCH_3$	D		121	Red needles	(60a)
2-$O_2NC_6H_4$	2-$O_2NC_6H_4$	$-(CH_2)_2OCOCH_3$	D		129	Red needles	(60a)
2-$CH_3OC_6H_4$	2-$CH_3OC_6H_4$	$-(CH_2)_2OCOCH_3$	D		97	Red needles, green reflex	(60a)
2-ClC_6H_4	2-ClC_6H_4	$-CH_2\underset{\displaystyle OCOCH_3}{\underset{\vert}{C}}HCH_3$	D		117	Orange plates	(60a)

* The corresponding tetrazolium salt has been described.
† Crude yield.

TABLE 11

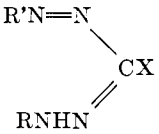
Formazans of the type



R	R'	Alkyl	Method of Synthesis	Yield	Melting Point	Appearance	Reference
				per cent	°C.		
C_6H_5	4-$O_2NC_6H_4$	CH_3	D	11	191-192	Bright red scales, gold reflex	(147)
4-$O_2NC_6H_4$	C_6H_5	CH_3	D	32	185	Brownish yellow	(147)
4-$O_2NC_6H_4$	4-$O_2NC_6H_4$	CH_3	D		193-194	Orange scales	(147)

TABLE 12

Formazans with simple substituent groups at position 3

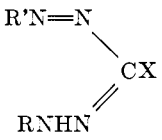


R	R'	X	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
C_6H_5	C_6H_5	NO_2	A1, A2	36-78	162-163	Red or maroon needles	(7, 18, 20, 22, 24, 54, 59, 72, 92, 104)
$2\text{-}CH_3C_6H_4$	$2\text{-}CH_3C_6H_4$	NO_2	A2		153-154	Maroon	(92)
$4\text{-}CH_3C_6H_4$	$4\text{-}CH_3C_6H_4$	NO_2	A2		160-162	Brick-red	(92)
$2,4\text{-}(CH_3)_2C_6H_3$	$2,4\text{-}(CH_3)_2C_6H_3$	NO_2	A2	21	145-148	Bright red	(129)
$\alpha\text{-}C_{10}H_7$	$\alpha\text{-}C_{10}H_7$	NO_2	A2		160-162	Chocolate-brown	(92)
$\beta\text{-}C_{10}H_7$	$\beta\text{-}C_{10}H_7$	NO_2	A2	63	198-200	Maroon	(92)
$4\text{-}C_6H_5C_6H_4$	$4\text{-}C_6H_5C_6H_4$	NO_2	A2		168-170	Maroon	(92)
$4\text{-}ClC_6H_4$	$4\text{-}ClC_6H_4$	NO_2	A2		188	Purplish red needles	(54)
$2,4\text{-}Cl_2C_6H_3$	$2,4\text{-}Cl_2C_6H_3$	NO_2	A2		189 d.	Purplish red needles	(54)
$4\text{-}BrC_6H_4$	$4\text{-}BrC_6H_4$	NO_2	A2		156-158	Brick-red	(92)
$4\text{-}O_2NC_6H_4$	$4\text{-}O_2NC_6H_4$	NO_2	A2		138-140	Maroon	(49, 92)
C_6H_5	$4\text{-}HO_3SC_6H_4$	NO_2	A1		213 d.	Red leaflets	(64)[a]
$4\text{-}H_2NSO_2C_6H_4$	$4\text{-}H_2NSO_2C_6H_4$	NO_2	A2		208-210	Orange-red	(92)
C_6H_5	C_6H_5	Cl	A2	40-45	153-154	Dark red needles, violet reflex	(74, 96)
$4\text{-}CH_3C_6H_4$	$4\text{-}CH_3C_6H_4$	Cl	A2	40-45	169-170	Dark red needles	(74)
$2,4\text{-}(CH_3)_2C_6H_3$	$2,4\text{-}(CH_3)_2C_6H_3$	Cl	A2		155	Dark orange	(75)
$\beta\text{-}C_{10}H_7$	$\beta\text{-}C_{10}H_7$	Cl	A2	70	213-214 206-207	Brown or dark yellow	(74, 96)
$2\text{-}ClC_6H_4$	$2\text{-}ClC_6H_4$	Cl	A2		179	Orange	(75)
$2\text{-}ClC_6H_4$	$4\text{-}O_2NC_6H_4$	Cl	A2	~100	199	Dark red	(75)
$4\text{-}O_2NC_6H_4$	$4\text{-}O_2NC_6H_4$	Cl	A2	Good	225	Yellow	(74)
C_6H_5	$4\text{-}HO_3SC_6H_4$	Cl	A2	35	<220	Dark blue prisms	(74)
$2\text{-}CH_3OOCC_6H_4$	$2\text{-}O_2NC_6H_4$	Cl	A2		209-210	Orange-red	(75)
$4\text{-}BrC_6H_4$	$4\text{-}BrC_6H_4$	Br	D		214-215	Minute orange crystals	(96)
C_6H_5	C_6H_5	I	D		142-143	Lustrous red prisms	(74)
C_6H_5	C_6H_5	OH	B,D,E		138 d.	Orange-yellow prisms	(9, 20, 51, 52, 63, 68, 85)[b]
C_6H_5	C_6H_5	SH	D,E	93[c]	168-169 d.	Blue-black plates or needles	(20, 35, 69 74, 96)[b]
$2\text{-}CH_3C_6H_4$	$2\text{-}CH_3C_6H_4$	SH	B		168 d.	Blue-black metallic needles	(71)
$4\text{-}CH_3C_6H_4$	$4\text{-}CH_3C_6H_4$	SH	B		105 d.	Dark blue flocks	(71)
$2,4\text{-}(CH_3)_2C_6H_3$	$2,4\text{-}(CH_3)_2C_6H_3$	SH	D		152-154		(129)
$\beta\text{-}C_{10}H_7$	$\beta\text{-}C_{10}H_7$	SH	D	21[d] [e]			(92, 96)
$4\text{-}BrC_6H_4$	$4\text{-}BrC_6H_4$	SH	D	3.9-5.6 [f]	145-147	Blue-black, coppery reflex	(92, 96)
C_6H_5	C_6H_5	NH_2	D	70	Variable	Dark red plates	(20, 74)[b][g]
C_6H_5	$x\text{-}ClC_6H_4$	NH_2	D		146 d.	Red leaflets, green reflex	(20)
C_6H_5	$x\text{-}BrC_6H_4$	NH_2	D		125-125.5	Dark red plates, green reflex	(20)[h]
C_6H_5	C_6H_5	SO_3H	G		Sinters at 192	Violet leaflets, blue reflex	(136)
C_6H_5	$4\text{-}BrC_6H_4$	SO_3H	G		Sinters at 196	Violet scales	(136)
C_6H_5	C_6H_5	SO_3H	D		118-120	Black	(95, 96)

[a] Potassium salt.
[b] The corresponding tetrazolium salt has been described.
[c] Yield of pure product, 40 per cent.
[d] Yield of pure product, 2.5 per cent.
[e] Crude yield.
[f] Yield of pure product.
[g] Hydrochloride, m.p. 141-142°C.
[h] Monoethanolate.

TABLE 13

Formazans with a carboxylic acid group or its derivative at position 3



R	R'	X	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
C_6H_5	C_6H_5	CN	A2	91*	158	Bronze leaflets, gold reflex	(158, 182, 193)†
4-BrC_6H_4	4-BrC_6H_4	CN	A2		259-260 d.	Red needles	(37)
2-HO-5-ClC_6H_3	2-HO-5-ClC_6H_3	CN	A2		210	Bordeaux-red needles	(201)
4-$O_2NC_6H_4$	4-$O_2NC_6H_4$	CN	A2	97*	247-248		(93)
2-$HOOCC_6H_4$	2-$HOOCC_6H_4$	CN	A2	65	225	Brownish red	(199)
C_6H_5	C_6H_5	COOH	A1, D		163 d.	Cherry-red needles, metallic reflex	(25, 26, 40, 110, 132, 137, 193, 195, 198)
C_6H_5	C_6H_5	$COOCH_3$	A1, A2	50	134-135	Red needles, blue reflex	(40, 132)
C_6H_5	C_6H_5	$COOC_2H_5$	A1, A2, B	80-100	117	Garnet-red needles or red prisms, blue reflex	(6, 25, 26, 106, 122, 132, 136, 146, 180, 198)
C_6H_5	4-$CH_3C_6H_4$	COOH	D		165-166	Brownish red leaflets	(134)
4-$CH_3C_6H_4$	C_6H_5	$COOCH_3$	A2		98	Red needles	(134)
C_6H_5	4-$CH_3C_6H_4$	$COOC_2H_5$	A1, A2		85	Garnet-red prisms	(134)
2-$CH_3C_6H_4$	2-$CH_3C_6H_4$	$COOC_2H_5$	A2	72-75	99-100		(146)
4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	COOH	D		182	Red rhombs	(195)
4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	COO-menthyl	A2		134-136	Dark red needles, blue reflex	(113)
2,4-$(CH_3)_2C_6H_3$	C_6H_5	$COOCH_6$	A1	60	138	Brownish red needles	(40)
4-$CH_3C_6H_4$	4-ClC_6H_4	COO-menthyl	A2		145-147		(113)
4-$CH_3C_6H_4$	4-BrC_6H_4	COO-menthyl	A2		149-151		(113)
2,4-$(CH_3)_2C_6H_3$	4-BrC_6H_4	$COOCH_3$	A1	Poor	177	Red needles	(40)
4-BrC_6H_4	4-BrC_6H_4	$COOC_2H_5$	A2	41	158-160	Bordeaux-red needles	(144)
C_6H_5	4-$O_2NC_6H_4$	$COOCH_3$	A1		182-183	Brownish red	(40)
2,4-$(CH_3)_2C_6H_3$	4-$O_2NC_6H_4$	$COOCH_3$	A1		208	Dark red prisms, steel-blue reflex	(40)
3-$O_2NC_6H_4$	3-$O_2NC_6H_4$	$COOC_2H_5$	A2		217	Red needles	(141)†
4-$O_2NC_6H_4$	4-$O_2NC_6H_4$	$COOC_2H_5$	A2	52*	202-204		(93)
4-HOC_6H_4	4-HOC_6H_4	COOH	C		186	Dark green tablets	(141)†
4-$C_2H_5OC_6H_4$	4-$C_2H_5OC_6H_4$	COOH	D		147-148	Black needles, blue reflex	(141)†
4-$C_2H_5OC_6H_4$	4-$C_2H_5OC_6H_4$	$COOC_2H_5$	A2	53	127-128	Dark red leaflets or needles, blue reflex	(141)†
C_6H_5	C_6H_5	—CH=CHCOOH	A2		199 d.	Reddish brown needles	(81)

TABLE 13—*Concluded*

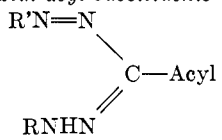
R	R′	X	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
C_6H_5	C_6H_5	—CH=CHCOOC$_2$H$_5$	A2,D	17	128	Red needles, gold reflex	(87, 144)
4-BrC$_6$H$_4$	4-BrC$_6$H$_4$	—CH=CHCOOC$_2$H$_5$	A2	62	150–151	Bordeaux-red needles	(144)
C_6H_5	C_6H_5	—CH=C(CH$_3$)COOH	A2	Good	193 d.	Dark needles	(86)
4-CH$_3$OC$_6$H$_4$	4-CH$_3$OC$_6$H$_4$	—(CH=CH)$_2$COOC$_2$H$_5$	A2		136-137	Dark red needles	(36a)
C_6H_5	C_6H_5	—COCOOH	A2	94	166	Red needles or golden needles	(18, 19, 97)
C_6H_5	C_6H_5	—COCOOCH$_3$	A2,D	72	124-125	Red needles	(18, 19, 97)
C_6H_5	C_6H_5	—COCOOC$_2$H$_5$	D	68	105-106	Ruby-red leaflets	(18, 19)
4-H$_2$NSO$_2$C$_6$H$_4$	4-H$_2$NSO$_2$C$_6$H$_4$	—COCOOH	A2		232	Orange	(125)
C_6H_5	C_6H_5	—CONHCOOC$_2$H$_5$	G		159 d.	Red plates	(195)
4-CH$_3$C$_6$H$_4$	4-CH$_3$C$_6$H$_4$	—CONHCOOC$_2$H$_5$	G		153	Red needles	(195)
2-O$_2$NC$_6$H$_4$	2-O$_2$NC$_6$H$_4$	—CONHCOOC$_2$H$_5$	G		105	Red plates, green reflex	(195)
4-O$_2$NC$_6$H$_4$	4-O$_2$NC$_6$H$_4$	—CONH$_2$	A2	89*	250		(93)

* Crude yield.

† The corresponding tetrazolium salt has been described.

TABLE 14

Formazans with acyl substituents at position 3



R	R'	Acyl	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
C_6H_5	C_6H_5	$COCH_3$	A1,A2	68-71	134-135	Ruby-red prisms, steel-blue reflex	(5, 14, 15, 17, 29, 57)*†
C_6H_5	4-$CH_3C_6H_4$	$COCH_3$	A1		126	Garnet-red leaflets, metallic reflex	(17)
4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	$COCH_3$	A2		153-154	Dark red needles, green reflex	(17)
α-$C_{10}H_7$	α-$C_{10}H_7$	$COCH_3$	A2		174.5-175	Dark green needles	(17)
C_6H_5	4-$O_2NC_6H_4$	$COCH_3$	A1		180	Ruby-red needles, steel-blue reflex	(15, 17)
C_6H_5	C_6H_5	COC_6H_5	A1,A2,G	24-41	141-142	Ruby-red needles, metallic reflex	(6, 27, 28)*‡
3-$O_2NC_6H_4$	3-$O_2NC_6H_4$	COC_6H_5	A2		210 d.	Dark red	(23)
2-HO-5-ClC_6H_3	2-HO-5-ClC_6H_3	COC_6H_4-2-COOH	A2		ca. 190 d.	Dark wine-red	(201)
C_6H_5	C_6H_5	$CH_2COC_6H_5$	A2		110	Red	(75)
2-$CH_3C_6H_4$	2-$CH_3C_6H_4$	$SO_2C_6H_5$	G		161	Dark red leaflets	(175)
2-$CH_3OC_6H_4$	2-$CH_3OC_6H_4$	$SO_2C_6H_5$	G		197	Red needles, green reflex	(175)
4-$O_2NC_6H_4$	4-$O_2NC_6H_4$	SO_2CH_3	A2	54	237-238		(93)

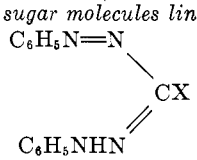
* The corresponding tetrazolium salt has been described.

† Acetyl derivative melts at 102°C.

‡ Acetyl derivative melts at 154°C.

TABLE 15

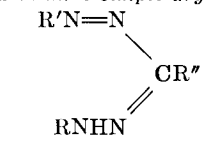
Formazans with sugar molecules linked to position 3



R	R'	X	Method of Synthesis	Yield	Melting Point	Appearance	Reference
				per cent	°C.		
C_6H_5	C_6H_5	Glucose	A1	33, 43	177-178	Red needles	(204)
C_6H_5	C_6H_5	Galactose	A1	61	167-168	Bronze flakes	(204)
C_6H_5	C_6H_5	Mannose	A1	43	174-175	Deep red needles, blue reflex	(204)
C_6H_5	C_6H_5	Pentaacetyl-galactose	A1,D	50, 78	142.5	Dark red needles	(207)
4-BrC_6H_4	C_6H_5	Galactose	A1	38	161-162	Brownish red needles	(207)
C_6H_5	4-C_6H_5CH=CHC_6H_4	Glucose	A1	Very small	183 d.	Dark red, green reflex	(208)

TABLE 16

Forazans with three simple aryl substituents



R	R'	R''	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
C_6H_5	C_6H_5	C_6H_5	A1	23–30	173.5	Red leaflets, green reflex	(2, 43, 82, 94, 100, 102, 106, 121, 133, 134, 197)[a]
C_6H_5	2-$CH_3C_6H_4$	C_6H_5	A1		154–155		(191)
C_6H_5	4-$CH_3C_6H_4$	C_6H_5	A1	63	155.5	Black needles, green reflex	(94, 134)[a]
4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	C_6H_5	A1		166	Black, green reflex	(134)
C_6H_5	2,4-$(CH_3)_2C_6H_3$	C_6H_5	A1		137		(191)
C_6H_5	4-iso-$C_3H_7C_6H_4$	C_6H_5	A1		113–114 173–174	Red prisms	(2, 191)[a]
C_6H_5	α-$C_{10}H_7$	C_6H_5	A1	80	173	Dark violet needles, green reflex	(64, 66, 83, 94, 150)
C_6H_5	β-$C_{10}H_7$	C_6H_5	A1	47	172	Dark violet needles, green reflex	(64, 83, 94, 150)
4-$CH_3C_6H_4$	α-$C_{10}H_7$	C_6H_5	A1		174	Violet, bronze reflex	(150)
4-$ClH_3C_6H_4$	β-$C_{10}H_7$	C_6H_5	A1		155	Violet-red needles, gold reflex	(150)
4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	4-$CH_3C_6H_4$					(101)
C_6H_5	4-$C_6H_5C_6H_4$	C_6H_5	A1	44	176	Deep red needles; dark purple, green reflex	(2, 82, 100, 184, 186)[a]
C_6H_5	C_6H_5	4-$C_6H_5C_6H_4$	A1	44	194–195	Deep red needles	(82, 100)[a]
4-$C_6H_5C_6H_4$	4-$C_6H_5C_6H_4$	C_6H_5	A1	13	172-173	Violet-red needles	(82, 100)[a]
4-$C_6H_5C_6H_4$	4-$C_6H_5C_6H_4$	4-$C_6H_5C_6H_4$	A1	24	214-216	Reddish black needles, metallic reflex	(100)[a]
C_6H_5	4-n-$C_{12}H_{25}C_6H_4$	C_6H_5	A1	83	85	Red needles, green reflex	(2)[a]
C_6H_5	2-$O_2NC_6H_4$	C_6H_5	A1		150	Ruby-red needles	(191)
C_6H_5	3-$O_2NC_6H_4$	C_6H_5	A1		180	Small needles	(191)
C_6H_5	4-$O_2NC_6H_4$	C_6H_5	A1	92	165–170 195	Greenish black needles	(2, 46, 191)[a]
C_6H_5	C_6H_5	3-$O_2NC_6H_4$	G		185	Pale reddish brown needles	(43)
C_6H_5	C_6H_5	4-$O_2NC_6H_4$	A1	40	204	Dark red needles, green reflex	(2)[a]

TABLE 16—*Continued*

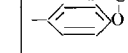
C_6H_5	C_6H_5	$2,4\text{-}(O_2N)_2C_6H_3$	A2		198	Crimson needles	(131)
$4\text{-}O_2NC_6H_4$	$\alpha\text{-}C_{10}H_7$	C_6H_5	A1	42	199–200	Dark red prisms, silky-violet needles, green reflex	(83, 150)
$4\text{-}O_2NC_6H_4$	$\beta\text{-}C_{10}H_7$	C_6H_5	A1		100	Violet scales, grey-green reflex	(150)
C_6H_5	$4\text{-}CH_3OC_6H_4$	$4\text{-}O_2NC_6H_4$	A1	86[b]	199	Brown needles	(183)[a]
C_6H_5	$4\text{-}ClC_6H_4$	C_6H_5	A1	59	117 164–165	Deep red needles	(2, 82)[a]
C_6H_5	C_6H_5	$4\text{-}ClC_6H_4$	A1		190	Red needles	(64)
C_6H_5	$4\text{-}BrC_6H_4$	C_6H_5	A1	50	191	Purplish brown, green reflex; red-brown needles, yellow reflex	(46, 94)
C_6H_5	$4\text{-}IC_6H_4$	C_6H_5	A1	45–60	195–196	Dark red needles, green reflex	(65, 70, 164)[a]
C_6H_5	$4\text{-}BrC_6H_4$	$4\text{-}BrC_6H_4$	A1	80	203	Black needles	(2)[a]
$2,4\text{-}Br_2C_6H_3$	C_6H_5	C_6H_5	B		173–174	Reddish brown needles	(55)
C_6H_5	$2,4,6\text{-}Br_3C_6H_2$	$4\text{-}BrC_6H_4$	A1	10	169	Purplish black needles	(2)[a]
$4\text{-}IC_6H_4$	$4\text{-}IC_6H_4$	C_6H_5	A1	42–51	169–170		(70)[a]
$2,4\text{-}I_2C_6H_3$	C_6H_5	C_6H_5	A1		186	Dark red needles	(65)
$2\text{-}CH_3\text{-}4\text{-}IC_6H_3$	C_6H_5	C_6H_5	A1		167	Black needles	(65)
$4\text{-}O_2NC_6H_4$	$4\text{-}IC_6H_4$	C_6H_5	A1	50	185–186	Reddish purple needles, gold reflex	(70)[a]
$4\text{-}BrC_6H_4$	$4\text{-}BrC_6H_4$	$2,4\text{-}(O_2N)_2C_6H_3$	A2		220	Crimson plates	(131)
$2,4\text{-}Cl_2C_6H_3$	$2,4\text{-}Cl_2C_6H_3$	$2,4\text{-}(O_2N)_2C_6H_3$	A2		206	Deep red powder	(131)
$2,4\text{-}Br_2C_6H_3$	$2,4\text{-}Br_2C_6H_3$	$2,4\text{-}(O_2N)_2C_6H_3$	A2		201	Deep red powder	(131)
$4\text{-}BrC_6H_4$	$4\text{-}BrC_6H_4$	C_6H_5	G		151	Garnet-red plates	(42)[c]
C_6H_5	$2\text{-}HOC_6H_4$	C_6H_5	A1	80	164–165	Violet, bronze reflex	(199)
C_6H_5	C_6H_5	$2\text{-}HOC_6H_4$	A1		164–165	Black powder	(64)
C_6H_5	$4\text{-}HOC_6H_4$	C_6H_5	C		153–155	Deep red	(180)[a]
C_6H_5	$4\text{-}CH_3OC_6H_4$	C_6H_5	A1	47–55	154	Green prisms	(180)[a]
C_6H_5	C_6H_5	$4\text{-}CH_3OC_6H_4$	G	<30	156–158	Brownish black needles, metallic reflex	(43, 101)
C_6H_5	$4\text{-}CH_3OC_6H_4$	$3,4\text{-}(CH_3O)_2C_6H_3$	A1	25	152–153	Reddish brown needles	(2)[a]
C_6H_5	C_6H_5		A1		156	Dark red needles	(64)
C_6H_5	$4\text{-}HOC_6H_4$	$4\text{-}O_2NC_6H_4$	C	Excellent[b]	194	Brownish red needles	(183)[a]
C_6H_5	$2\text{-}HOOCC_6H_4$	C_6H_5	A1	75	181–182	Orange-red	(199)
$3\text{-}HOOCC_6H_4$	C_6H_5	C_6H_5	A1		202	Reddish black needles	(191)[a]
C_6H_5	$4\text{-}(C_2H_5)_2N(CH_2)_2$ $\vert$ $OOCC_6H_4$	C_6H_5	A1	64	172	Dark red prisms, coppery reflex	(2)[d]
$2\text{-}HOOCC_6H_4$	$2\text{-}HOOCC_6H_4$	C_6H_5	A1	75–80	198	Red needles	(199)[c]
$3\text{-}HOOCC_6H_4$	$2\text{-}HOOCC_6H_4$	C_6H_5	A1	64	225	Pale red powder	(191)

TABLE 16—*Continued*

R	R'	R''	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
3-HOOCC$_6$H$_4$	3-HOOCC$_6$H$_4$	C$_6$H$_5$	A1	66	214		(191)
3-HOOCC$_6$H$_4$	4-HOOCC$_6$H$_4$	C$_6$H$_5$	A1	82	218		(191)
C$_6$H$_5$	C$_6$H$_5$	4-CNC$_6$H$_4$	A1	64.5	215	Red needles, metallic reflex	(2)[a]
C$_6$H$_5$	4-CNC$_6$H$_4$	4-CNC$_6$H$_4$	A1	80	229 d.	Purple needles, green reflex	(2)
4-CNC$_6$H$_4$	4-CNC$_6$H$_4$	4-CNC$_6$H$_4$	A1	Very small	255–257	Dark red, green reflex	(126a)
3-HOOCC$_6$H$_4$	3-O$_2$NC$_6$H$_4$	C$_6$H$_5$	A1	92	185	Red	(191)
3-HOOCC$_6$H$_4$	2-ClC$_6$H$_4$	C$_6$H$_5$	A1	82	217	Reddish black needles	(191)
2-HOOC-4-HO$_3$SC$_6$H$_3$	2-HOC$_6$H$_4$	C$_6$H$_5$	A1				(56)
C$_6$H$_5$	4-CH$_3$CONHC$_6$H$_4$	C$_6$H$_5$	A1	55	212-213	Reddish black	(187)[a]
C$_6$H$_5$	C$_6$H$_5$	4-CH$_3$CONHC$_6$H$_4$	A1	53	206–207		(2)[a]
C$_6$H$_5$	2-Cl-4-CH$_3$CONHC$_6$H$_3$	C$_6$H$_5$	A1	76	184–185	Black prisms, red reflex	(2)[a]
C$_6$H$_5$	3-Cl-4-CH$_3$CONHC$_6$H$_3$	C$_6$H$_5$	A1	44	184–185	Purple needles, yellow reflex	(2)[a]
C$_6$H$_5$	2-O$_2$N-4-ClH$_3$CONHC$_6$H$_3$	C$_6$H$_5$	A1	57	210–212	Purplish brown plates	(2)[a]
C$_6$H$_5$	3-HO-4-CH$_3$CONHC$_6$H$_3$	C$_6$H$_5$	D		188–190		(2)
C$_6$H$_5$	3-CH$_3$COO-4-CH$_3$CONH C$_6$H$_5$	C$_6$H$_5$	A1	39	217-218	Reddish purple needles	(2)[a]
C$_6$H$_5$	4-CH$_3$CONHC$_6$H$_4$	4-CH$_3$CONHC$_6$H$_4$	A1	17	236–237		(2)
C$_6$H$_5$	4-(CH$_3$)$_2$NC$_6$H$_4$ COCH$_3$	4-ClC$_6$H$_4$	D		198	Black needles, steel-blue reflex	(126)
C$_6$H$_5$	4-(*n*-C$_{12}$H$_{25}$N)C$_6$H$_4$ COCH$_3$	C$_6$H$_5$	A1		Oil	Red	(2)[a]
C$_6$H$_5$	4-CH$_3$CONH(CH$_2$)$_{12}$NC$_6$H$_4$	C$_6$H$_5$	A1		Gum	Red	(2)[a]
C$_6$H$_5$	4-HO$_3$SC$_6$H$_4$	C$_6$H$_5$	A1		ca. 165 d.	Red needles	(66)[f]
4-HO$_3$SC$_6$H$_4$	C$_6$H$_5$	C$_6$H$_5$	A1		ca. 135 d.	Dark red needles	(66)[f]
C$_6$H$_5$	C$_6$H$_5$	3-HO$_3$SC$_6$H$_4$	A1			Red needles	(66)[f]
C$_6$H$_5$	α-(4-HO$_3$S)C$_{10}$H$_6$	C$_6$H$_6$	A1				(64)[f]
C$_6$H$_5$	4-HO$_3$SC$_6$H$_4$	4-ClC$_6$H$_4$	A1			Red needles	(64)[g]
C$_6$H$_5$	4-IC$_6$H$_4$	3-HO$_3$SC$_6$H$_4$	A1			Red powder	(65)[f]
C$_6$H$_5$	4-HO$_3$SC$_6$H$_4$	2-HOC$_6$H$_4$	A1			Black needles	(64)[g]
C$_6$H$_5$	α-(4-HO$_3$S)C$_{10}$H$_6$	4-ClC$_6$H$_4$	A1			Dark red leaflets	(64)[g]
C$_6$H$_5$	4-(C$_2$H$_5$)$_2$N(CH$_2$)$_2$ CH$_3$CH NHSO$_2$C$_6$H$_4$	C$_6$H$_5$	A1	47	194 d.	Red prisms	(2)[d]

TABLE 16—*Concluded*

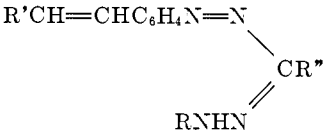
C_6H_5	4-$HO_2SC_6H_4$		A1			Dark red leaflets	(64)[g]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$ I^-	C_6H_5	A1	27	179–180	Purplish brown prisms, red reflex	(126)[a]
C_6H_5	C_6H_5	4-$(CH_2)_3\overset{+}{N}C_6H_4$ Cl^-	D	34	177-179 d.	Reddish brown prisms, gold reflex	(126)[a][b]
C_6H_5	C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$ I^-	A1	24	170–171	Dark red prisms, gold reflex	(126)[e]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	A1	18	184–186 d.	Red needles, gold reflex	(126)[a][i]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	4-ClC_6H_4	A1	42	197-198	Dark red plates, yellow reflex	(126)[a][i]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	4-$O_2NC_6H_4$	A1	Poor	184–185	Red microcrystals	(126)[a]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	4-$HOOCC_6H_4$	A1	22–32	223 d.	Red needles, green reflex	(126)[a]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	4-$ClH_2COOC_6H_4$	D	58, 84	164	Dark purple	(126)[e]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$ $CH_3SO_4^-$	4-$CH_3COOC_6H_4$	A1	48	206–207	Red plates, purple reflex	(126)
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	4-HOC_6H_4	D	69	194–195 d.	Reddish purple, red reflex	(126)[a]
4-$CH_3CONHC_6H_4$	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	C_6H_5	A1	41	188	Reddish black, green reflex	(126)[a]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	4-$CH_3CONHC_6H_4$	A1	38	173–174	Purple plates	(126)[a][k]
C_6H_5	4-$CH_3CONHC_6H_4$	4-$(CH_3)_3\overset{+}{N}C_6H_4$ I^-	A1	22	175–176	Reddish black needles, green-yellow reflex	(126)[a][h]
4-$CH_3CONHC_6H_4$	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	4-$ClH_2CONHC_6H_4$	A1	38	179–180	Dark red needles, green reflex	(126)[a][i]
4-$CH_3CONHC_6H_4$	4-$CH_3CONHC_6H_4$	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	A1	4	191	Dark purple needles, gold reflex	(126)[l]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	4-CNC_6H_4	A1	52	170–172	Brownish red needles, gold reflex	(126)[a][c]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	4-$ClH_2OOCC_6H_4$	A1	33	180–182	Dark red prisms, greenish gold reflex	(126)[a][c]
C_6H_5	2-$C_6H_5CH_2OC_6H_4$	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	A1	29	164–166	Purplish red needles, gold reflex	(126)[a]
4-$ClH_2C_6H_4$	4-ClC_6H_4	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	A1	33	180 d.	Dark red needles, gold reflex	(126)[c]
C_6H_5	2-ClC_6H_4	4-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	A1	80[b]	178	Red needles, gold reflex	(126)[a][c]
C_6H_5	C_6H_6	3-$(CH_3)_3\overset{+}{N}C_6H_4$ Cl^-	A1	13	174–175	Dark red prismatic needles, gold reflex	(126)[h]
C_6H_5	C_6H_6	4-$(n\text{-}C_3H_7(CH_3)_2)\overset{+}{N}C_6H_4$ Cl^-	A1	Poor	165	Dark red needles, gold reflex	(126)[L]

See also table 18.

[a] The corresponding tetrazolium salt has been described.
[b] Crude yield.
[c] *N*-Benzoyl derivative.
[d] Hydrochloride.
[e] Monohydrate.
[f] Sodium salt.
[g] Potassium salt.
[l] Dihydrate.
[i] Trihydrate.
[i] Monomethanolate.
[k] Monoethanolate.
[l] Sesquihydrate.

TABLE 17

Formazans with a styryl group attached to one nitrogen atom



R	R'	R"	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
C_6H_5	C_6H_5	CH_3	A1	68	160–162	Dark orange plates	(127)*
C_6H_5	4-$O_2NC_6H_4$	CH_3	A1	16	182-183	Dark orange plates	(127)*
C_6H_5	4-$CH_3CONHC_6H_4$	CH_3	A1	12	203	Red prisms	(127)
C_6H_5	C_6H_5	C_6H_5	A1	74	185	Purple needles, green reflex	(127)*
C_6H_5	C_6H_5	4-BrC_6H_4	A1	47	170–171	Purple needles	(127)*
C_6H_5	C_6H_5	4-$CH_3OC_6H_4$	A1	83	157-158	Purple plates	(127)*
C_6H_5	4-BrC_6H_4	C_6H_5	A1	33	186-187	Purple rods	(127)*
C_6H_5	4-$O_2NC_6H_4$	C_6H_5	A1	33	185-186	Purple needles, golden reflex	(127)*
C_6H_5	4-HOC_6H_4	C_6H_5	A1	32	175-176	Purple prisms	(127)*†
C_6H_5	4-$CH_3CONHC_6H_4$	C_6H_5	A1	14	208–209	Purple needles, green reflex	(127)*
$C_6H_5N{=}NC_6H_4$	C_6H_5	C_6H_5	A1	47		Purplish black, red reflex	(127)*
$C_6H_5N{=}NC_6H_4$	C_6H_5	4-$CH_3COOC_6H_4$	A1	40	220–221	Purple prisms	(127)*
$C_6H_5N{=}NC_6H_4$	C_6H_5	4-$HOOCC_6H_4$	A1	33	239-240	Black microcrystalline solid	(127)*

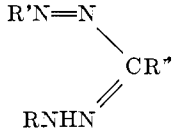
See also tables 15 and 19.

* The corresponding tetrazolium salt has been described.

† Hemihydrate.

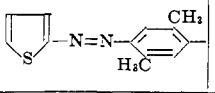
TABLE 18

Formazans with a phenylazo group



R	R'	R"	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
C_6H_5	4-$C_6H_5N{=}NC_6H_4$	CH_3	A1	28	115	Dark red	(117)*†
C_6H_5	4-$C_6H_5N{=}NC_6H_4$	C_6H_5	A1	50	182 d.	Purplish black	(117)*
C_6H_5	4-$C_6H_5N{=}NC_6H_4$	4-HOC_6H_4	A1	50	180	Dark purple needles	(117)*‡
C_6H_5	4-$C_6H_5N{=}NC_6H_4$	4-$CH_3COOC_6H_4$	A1	50	191	Dark purple needles	(117)*
4-$HOOCC_6H_4$	4-$C_6H_5N{=}NC_6H_4$	C_6H_5	A1	10	209	Black plates, green reflex	(117)*
4-$CH_3CONHC_6H_4$	4-$C_6H_5N{=}NC_6H_4$	C_6H_5	A1	26	215	Purple microcrystals	(117)*

TABLE 18—*Concluded*

R	R'	R''	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
C_6H_5	C_6H_5	$C_6H_5N{=}N-$	A1, A2	20–30	162	Dark red leaflets, bronze reflex	(6, 7, 18, 19, 25, 26, 132, 137, 197, 204)*§
$4\text{-}CH_3C_6H_4$	C_6H_5	$C_6H_5N{=}N-$	G		174-175	Dark red leaflets, bronze reflex	(134)
$4\text{-}ClC_6H_4$	$4\text{-}C_6H_5N{=}NC_6H_4$	C_6H_5	A1	18	168-170	Purplish black	(117)*
C_6H_5	$4\text{-}4'\text{-}CH_3C_6H_4$ | $N{=}NC_6H_4$	C_6H_5	A1	53	186-188	Black needles, reddish purple reflex	(117)*
C_6H_5	$4\text{-}4'\text{-}ClC_6H_4$ | $N{=}NC_6H_4$	C_6H_5	A1	11.5	194.5–195	Dark purple needles	(117)*
C_6H_5	$4\text{-}4'\text{-}HOC_6H_4$ | $N{=}NC_6H_4$	C_6H_5	A1	28	198–200	Dark purple needles, red reflex	(117)*
C_6H_5	$4\text{-}2'\text{-}Cl\text{-}4'\text{-}HOC_6H_3$ | $N{=}NC_6H_4$	C_6H_5	A1	27	149-150	Dark purple plates, yellow reflex	(117)*
C_6H_5	$4\text{-}3'\text{-}Cl\text{-}4'\text{-}HOC_6H_3$ | $N{=}NC_6H_4$	C_6H_5	A1	8	205-210 d.	Black, yellow-green reflex	(117)*
C_6H_5	$4\text{-}4'\text{-}CH_3CONHC_6H_4$ | $C_6H_4N{=}N$	C_6H_5	A1	35	216	Dark red prisms	(117)
C_6H_5	$4\text{-}4'\text{-}(CH_3)_2NC_6H_4$ | $N{=}NC_6H_4$	C_6H_5	A1	23	182	Dark purple	(117)
C_6H_5	$4\text{-}4'\text{-}O_2NC_6H_4$ | $N{=}NC_6H_4$	C_6H_5	A1	51	205-206 d.	Purplish black prisms, gold reflex	(117)*
C_6H_5	$2,5\text{-}(CH_3)_2C_6H_2-$ | $4\text{-}C_6H_5N{=}N$	C_6H_5	A1	50	197 d.	Dark purple flat needles, green reflex	(117)*
C_6H_5	$\alpha\text{-}(4\text{-}C_6H_5N{=}N)C_{10}H_6$	C_6H_5	A1	9	200	Indigo-blue needles	(117)*
$4\text{-}CH_3CONHC_6H_4$	$4\text{-}4'\text{-}HOC_6H_4N{=}NC_6H_4$	$4\text{-}CH_3CONHC_6H_4$ + Cl^-	A1	Small	188	Dark purple	(117)¶
C_6H_5	$4\text{-}4'\text{-}HOC_6H_4N{=}NC_6H_4$	$4\text{-}(CH_3)_3NC_6H_4$ Cl^-	A1	8.5	172-173 d.	Purplish black prisms	(126)*
C_6H_5		C_6H_5	A1	25	216 d.	Violet needles	(117)

See also tables 17, 19, and 20.

* The corresponding tetrazolium salt has been described.

† Monohydrate.

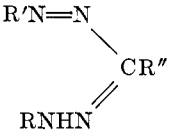
‡ Hemimethanolate.

§ Acetyl derivative melts at 190°C.

¶ Dimethanolate.

TABLE 19

Formazans containing a heterocyclic group



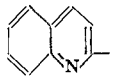
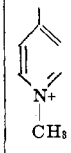
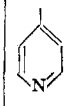
R	R'	R''	Method of Synthesis	Yield	Melting Point	Appearance	Reference
				per cent	°C.		
C_6H_5	C_6H_5		A1	14	118	Purple prisms, gold reflex	(130)*
	4-ClC_6H_4		A1	64	114	Red needles	(154)
	4-ClC_6H_4		A1	61	108	Dark red needles	(154)*
C_6H_5	3-$CF_3C_6H_4$		A1	57	128	Wine-red needles	(154)*
C_6H_5	C_6H_5		A1	46	185-187	Red plates, yellow reflex	(130)*
C_6H_5	C_6H_5	 I^-	D	92	233-234 d.	Red needles, green reflex	(130)*
		Cl^-		80	210-212	Red needles, green reflex	(130)
		$CH_3SO_4^-$		81	204-205	Red needles, blue reflex	(130)
C_6H_5		C_6H_5	A1	53	168-169	Purple prisms, yellow reflex	(2)*
C_6H_5	4-ClC_6H_4		A1	40	170-172	Red plates, gold reflex	(130)
	4-ClC_6H_4	C_6H_5	A1	24	108	Reddish black needles	(154)*
	4-ClC_6H_4	2-ClC_6H_4	A1	24	105	Deep red	(154)*
	4-ClC_6H_4	2-$O_2NC_6H_4$	A1	21	146	Black needles	(154)*
	4-ClC_6H_4	2-HOC_6H_4	A1	41	194	Brownish red	(154)*

TABLE 19—*Continued*

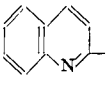
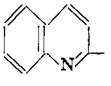
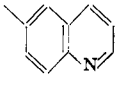
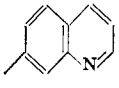
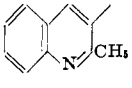
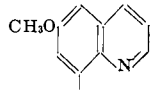
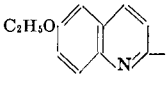
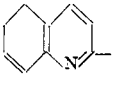
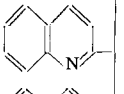
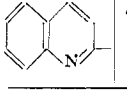
R	R′	R″	Method of Synthesis	Yield	Melting Point	Appearance	Reference
				per cent	°C.		
	4-ClC_6H_4	4-$CH_3OC_6H_4$	A1	44	145	Brownish red needles	(154)*
	4-ClC_6H_4		A1	59	149	Reddish brown needles	(154)*
C_6H_5	4-$CH_3OC_6H_4$		A1	95	158	Red needles, blue reflex	(130)*
C_6H_5	4-$O_2NC_6H_4$		A1	76	205–207 d.	Dark red prisms, blue reflex	(126a)
C_6H_5	C_6H_5		G		185	Dark reddish brown, green reflex	(61)
C_6H_5	2-$HOOCC_6H_4$		A1	65	198 d.	Red	(168)
C_6H_5		C_6H_5	A1	48	174	Greenish black needles	(154)*
C_6H_5		C_6H_5	A1		168	Dark red needles	(154)*
C_6H_5		C_6H_5	A1		211	Reddish black needles	(154)
C_6H_5		C_6H_5	A1	20	178–179	Dark purple microcrystals	(2)*
C_6H_5		C_6H_5	A1		145–146	Blue-black needles	(154)
4-BrC_6H_4	4-BrC_6H_4		A2	79†	199–200	Dark red	(156)*
	C_6H_5	C_6H_5	A1		160		(163)
	4-ClC_6H_4	C_6H_5	A1	47	176	Reddish black needles, green reflex	(154)*

TABLE 19—*Continued*

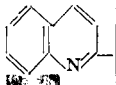
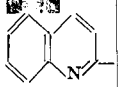
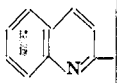
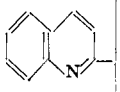
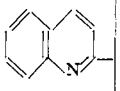
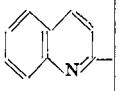
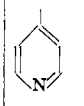
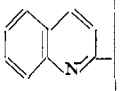
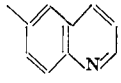
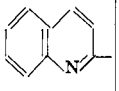
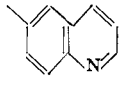
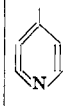
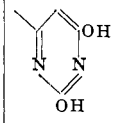
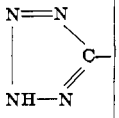
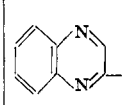
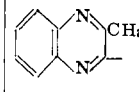
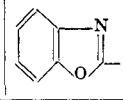
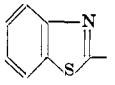
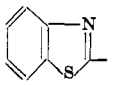
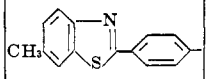
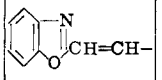
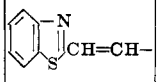
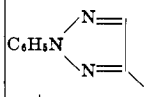
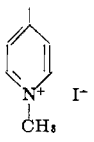
R	R'	R''	Method of Synthesis	Yield	Melting Point	Appearance	Reference
				per cent	°C.		
	4-ClC_6H_4	2-ClC_6H_4	A1	21	150	Orange-red	(154)*
	4-ClC_6H_4	2-$O_2NC_6H_4$	A1	49	188	Red leaflets, green reflex	(154)*
	4-ClC_6H_4	2-HOC_6H_4	A1	31	179	Reddish black needles	(154)*
	4-ClC_6H_4	4-$CH_3OC_6H_4$	A1	29	172	Reddish black, green reflex	(154)*
	4-ClC_6H_4		A1	85	82	Red needles	(154)*
	4-ClC_6H_4		A1	78	208	Red needles	(154)*
			A1	30	103	Red needles	(154)*
			A1		221	Red needles	(154)*
C_6H_5	C_6H_5		A1	76	266	Dark red needles	(118)*
	C_6H_5	C_6H_5	A1	50–60	147		(163)
4-BrC_6H_4	4-BrC_6H_4		A2	78†	208–212	Red-violet	(156)*
4-ClC_6H_4	4-ClC_6H_4		A2	44	240	Lemon-yellow needles	(156)
4-BrC_6H_4	4-BrC_6H_4		A2	76†	218–220	Bordeaux-red needles	(156)*

TABLE 19—*Concluded*

R	R'	R''	Method of Synthesis	Yield	Melting Point	Appearance	Reference
				per cent	°C.		
4-BrC_6H_4	4-BrC_6H_4		A2	62†	214–215	Orange-red needles	(156)*
C_6H_5	2-$HOOCC_6H_4$		A1		246–247 d.	Dark red	(168a)
C_6H_5		C_6H_5	A1		158–159	Black needles	(154)
C_6H_5		C_6H_5	A1		197	Deep violet microcrystals	(154)*
C_6H_5	C_6H_5		A2		148	Deep violet	(156)
4-BrC_6H_4	4-BrC_6H_4		A2	46	203	Deep violet needles	(156)
C_6H_5	C_6H_5		A1	59	163–164	Red needles, gold reflex	(130)*
C_6H_5	4-$C_6H_5N{=}NC_6H_4$		A1	39	194	Red needles, green reflex	(130)*
C_6H_5	4-$C_6H_5CH{=}CHC_6H_4$		A1	40	184–187	Red needles, green reflex	(130)*
C_6H_5	4-$C_6H_5CH{=}CHC_6H_4$		A1	84	200 d.	Red needles, blue reflex	(130)

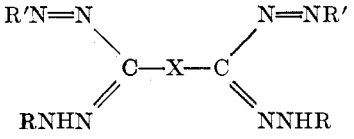
See also tables 18, 22, 24, and 25.

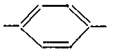
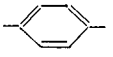
* The corresponding tetrazolium salt has been described.

† Crude yield.

TABLE 20

Bisformazans of the type



R	R'	X	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
C_6H_5	C_6H_5		A2	10–14	226 d.	Greenish brown leaflets, metallic reflex	(16, 19, 82, 100)* ‡
C_6H_5	C_6H_5	$-(CH_2)_2-$	A1	63	148–149	Red needles	(82, 100)*
C_6H_5	$4\text{-}C_6H_5N{=}NC_6H_4$	$-(CH_2)_2-$	A1	29	136–138	Purple	(117)*
C_6H_5	C_6H_5	$-(CH_2)_6-$	A1		129–132 d.	Dark red	(2)*
C_6H_5	$4\text{-}C_6H_6N{=}NC_6H_4$	$-(CH_2)_6-$	A1	39	161–162	Purplish black prisms	(117)* §
C_6H_5	C_6H_5		A1	90†	208–210	Violet-red	(100)*
C_6H_5	$4\text{-}C_2H_5OOCC_6H_4$		A1	46	224–225		(82, 100)*

* The corresponding tetrazolium salt has been described.

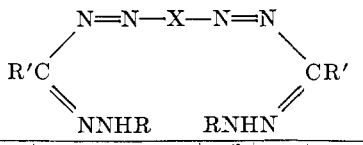
† Crude yield.

‡ Hydrochloride, m.p. 245°C. (d.).

§ One molecule of cyclohexane of crystallization.

TABLE 21

Bisformazans of the type



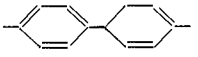
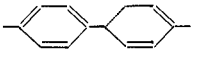
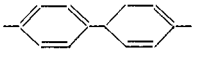
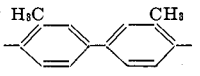
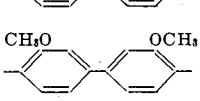
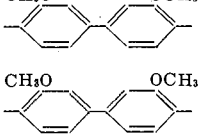
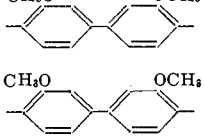
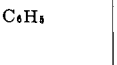
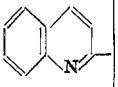
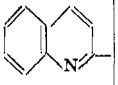
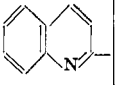
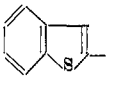
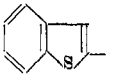
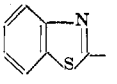
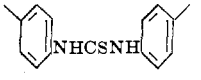
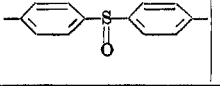
R	X	R'	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
C_6H_5		CH_3	A1	36	197	Deep red	(100)*
C_6H_5		C_6H_5	A1	76	219-220 d.	Dark violet needles	(163a, 186)*
C_6H_5			A1	33†	233	Black needles, gold reflex	(154)
C_6H_5		C_6H_5	A1	43	241 d.	Black needles, gold reflex	(163a)
C_6H_5		C_6H_5	A1	68	244	Blue-black needles, gold reflex	(153, 160, 163a)*
C_6H_5		$2\text{-}HOC_6H_4$	A1		233	Black needles	(153)*
C_6H_5		$4\text{-}CH_3OC_6H_4$	A1		202	Black needles, gold reflex	(153)*
C_6H_5		$3,4\text{-}(CH_3O)_2C_6H_3$	A1	71†	202	Black needles, gold reflex	(153)*
C_6H_5			A1	70†	223	Black, gold reflex	(153, 154)*
C_6H_5			A1	54	230	Blue-black needles, gold reflex	(153, 154)*
			A1	37	200	Black, yellow reflex	(154)
			A1	43	222	Brownish black needles	(154)
C_6H_5			A1	30	216	Green needles	(154)*
		C_6H_5	A1	23	218	Black needles, green reflex	(154)*

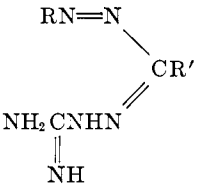
TABLE 21—*Concluded*

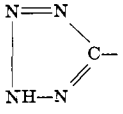
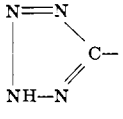
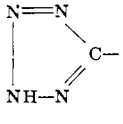
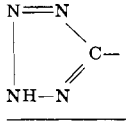
R	X	R'	Method of Synthesis	Yield	Melting Point	Appearance	References
				per cent	°C.		
C_6H_5	CH_3O OCH_3		A1	42	272-274	Black needles, green reflex	(153, 154)*
	CH_3O OCH_3	$2\text{-}ClC_6H_4$	A1		187	Reddish black leaflets	(154)*
	CH_3O OCH_3	$2\text{-}O_2NC_6H_4$	A1	26	205	Black needles	(154)*
	CH_3O OCH_3		A1	32	214	Dark green needles	(154)*
	CH_3O OCH_3		A1	27	217	Black needles, green reflex	(154)*
	CH_3O OCH_3	C_6H_5	A1	50	153	Black	(154)*
	CH_3O OCH_3	$2\text{-}ClC_6H_4$	A1	13	161	Black needles, green reflex	(154)*
	CH_3O OCH_3	$2\text{-}O_2NC_6H_4$	A1	53	203	Black, gold reflex	(154)*
C_6H_5	CH_3O OCH_3		A1	57	248	Black needles, gold reflex	(153, 154)*
	CH_3O OCH_3		A1	36	241	Black needles, violet reflex	(154)*
C_6H_5	CH_3O OCH_3		A1		226	Black needles	(154)
C_6H_5		C_6H_5	A1	9	186.5-187	Violet-red needles	(163a)*
C_6H_5		C_6H_5	A1	32	197-199	Reddish brown needles, green-gold reflex	(163a)*‡

* The corresponding tetrazolium salt has been described.
† Crude yield.
‡ Monohydrate.

TABLE 22

Guanazyls of the formula

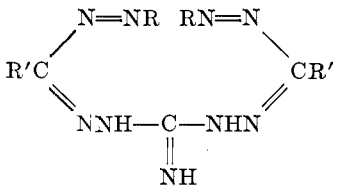


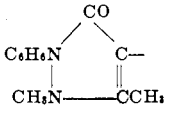
R	R′	Method of Synthesis	Yield	Melting Point	Appearance	Reference
			per cent	°C.		
3-$O_2NC_6H_4$	CH_3	F		222	Bright red needles or dark red prisms	(189)
C_6H_5	C_6H_5	F	61*	199	Orange-yellow prisms	(181)
3-$O_2NC_6H_4$	C_6H_5	F		206	Orange-red needles	(181)
3-$H_2NC_6H_4$	C_6H_5	D		193	Brownish yellow needles	(181)
C_6H_5	4-$O_2NC_6H_4$	F		209	Yellowish brown powder	(181)
C_6H_5	2-HOC_6H_4	F		191–192	Light yellow leaflets	(185)
		F		149		(163)†
	4-$CH_3OC_6H_4$	F		146		(163)†
	4-iso-$C_3H_7C_6H_4$	F		128		(163)
	4-ClC_6H_4	F		146		(163)

* Crude yield.
† Hydrate

TABLE 23

Guanazyls of the formula

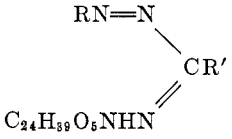


R	R′	Method of Synthesis	Melting Point	Appearance	Reference
			°C.		
C_6H_5	C_6H_5	F	137-138	Red	(161)
C_6H_5	3-$O_2NC_6H_4$	F	148-149	Dark red	(161)
C_6H_5	4-$HO_3SC_6H_4$	F		Deep purplish red	(161)* †
2-$O_2NC_6H_4$	C_6H_5	F	115		(163)
4-$O_2NC_6H_4$	C_6H_5	F	175		(163)
2-CH_3-6-$O_2NC_6H_3$	C_6H_5	F	94		(163)‡
4-CH_3-2-$O_2NC_6H_3$	C_6H_5	F	108		(163)
β-$C_{10}H_7$	C_6H_5	F	163		(163)
2-CH_3-4-ClC_6H_3	C_6H_5	F	63		(163)
	C_6H_5	F	154		(163)§

* Potassium salt.
† *p*-Toluidine salt, m.p. 227-229°C.
‡ Dihydrate.
§ Hydrate.

TABLE 24

Cholyl compounds of the formula



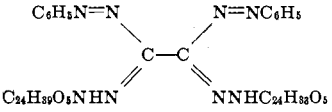
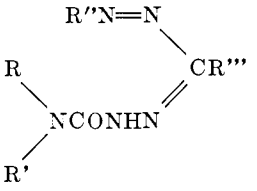
R	R′	Method of Synthesis	Melting Point	References
			°C.	
C_6H_5	H	G	47	(50)
C_6H_5	*n*-C_3H_7	G	Liquid	(50)
C_6H_5	C_6H_5	G	111	(50)
C_6H_5	$C_6H_5CH_2$	G	Liquid	(50)
C_6H_5		G	58	(50)
		G	53	(50)

TABLE 25

Semicarbazide derivatives

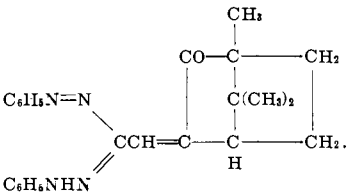
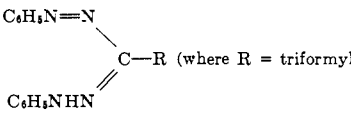


R	R'	R''	R'''	Method of Synthesis	Yield	Melting Point	Appearance	Reference
					per cent	°C.		
C_6H_5	C_6H_5	C_6H_5	CH_3	G	30.5	151	Orange-red needles	(155)
C_6H_5	C_6H_5	C_6H_5	C_6H_5	G	82	173-174	Ruby-red scales	(155)
C_6H_5	C_6H_5	C_6H_5	2-HOC_6H_4	G	55	174	Brick-red needles	(155)*
C_6H_5	C_6H_5	C_6H_5		G	30	153	Dark red	(155)
β-$C_{16}H_7$	β-$C_{10}H_7$	C_6H_5	C_6H_5	G		175	Reddish black needles	(155)
C_6H_5	β-$C_{10}H_7$	C_6H_5	C_6H_5	G	100	172	Reddish brown needles	(155)

* This compound is of doubtful authenticity.

TABLE 26

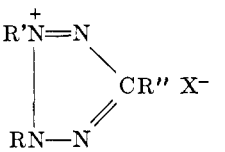
Miscellaneous compounds

	Method of Synthesis	Melting Point	Appearance	Reference
		°C.		
	G	152-154	Red	(159)
	G	Oil	Deep red	(79)*

* The corresponding tetrazolium salt has been described.

TABLE 27

Tetrazolium salts with alkyl groups at position 5



R	R'	R''	Anion X	Method of Synthesis*	Yield	Melting Point	Appearance	References
C_6H_5	C_6H_5	H	Cl	A	85	268 d.	Colorless needles	(140)†
C_6H_5	C_6H_5	H	I			237	Colorless needles	(140)
C_6H_5	C_6H_5	CH_3	Cl	C,E,G	81‡	271 d.	Colorless needles	(34, 99, 102, 106, 108, 191)
C_6H_5	C_6H_5	CH_3	Br	G		263-264	Colorless needles	§
C_6H_5	4-HOC_6H_4	CH_3	Cl	C		261	Colorless needles	(103)
C_6H_5	2-$HOOCC_6H_4$	CH_3	Cl	C		242		(103)
C_6H_5	4-$HOOCC_6H_4$	CH_3	Cl	C	Good	268		(103)
C_6H_5	4-$NH_2SO_2C_6H_4$	CH_3	Cl	C		198	Pale yellow	(98)
C_6H_5	C_6H_5	C_2H_5	Cl	C	60-80	245	Colorless prisms	(34, 38, 82)
C_6H_5	4-$NH_2SO_2C_6H_4$	*n*-C_3H_7	Cl	C	79	179	Pale yellow	(98)
C_6H_5	C_6H_5	iso-C_3H_7	Cl	C	60-80	245-250		(38)
C_6H_5	C_6H_5	iso-C_4H_9	Cl	C	60-80	220-230		(38)
C_6H_5	C_6H_5	*n*-C_5H_{11}	Cl	C		210-214		(38)
C_6H_5	C_6H_5	*n*-C_6H_{13}	Cl	C	72	220 d.	Colorless needles	(106)
C_6H_5	4-$NH_2SO_2C_6H_4$	*n*-C_6H_{13}	Cl	C	83	147	Yellow	(98)
C_6H_5	2-$HOOCC_6H_4$	*n*-C_7H_{15}	Cl	C	54‡	228-230		(103, 194)
C_6H_5	4-$NH_2SO_2C_6H_4$	*n*-C_7H_{15}	Cl	C	83	142		(98)
C_6H_5	C_6H_5	*n*-$C_{11}H_{23}$	Cl	C	83	141		(106)
C_6H_5	4-BrC_6H_4	*n*-$C_{11}H_{23}$	Cl	C	74‡	60		(106)
C_6H_5	4-$NH_2SO_2C_6H_4$	*n*-$C_{11}H_{23}$	Cl	C	100	135	Yellow	(98)
C_6H_5	α-$C_{10}H_7$	*n*-$C_{11}H_{23}$	Cl	C	Good		Glass	(106)

* For an explanation of these symbols see page 434.

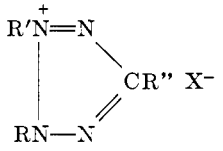
† Also OH, NO_6.

‡ Crude yield.

§ Platinichloride, m.p, 238-239°C.

TABLE 28

Tetrazolium salts with simple substituent groups at position 5



R	R'	R''	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	References
C_6H_5	C_6H_5	OH	Betaine	A,B	80	174 d.	Colorless needles or leaflets	(8, 9, 20, 53, 63, 74)
C_6H_5	C_6H_5	OH	Cl	A		193		(74)*
C_6H_5	C_6H_5	SH	Betaine	H	20	180.5 d.	Pale yellow needles	(20)
C_6H_5	C_6H_5	NH_2	Cl	A	72	269	Colorless	(20)
C_6H_5	C_6H_5	$-\bar{N}NO$	Betaine	B	58	177	Orange-yellow cubes	(20)
C_6H_5	C_6H_5	$-\bar{N}NO$	Cl			168	Colorless	(20)
C_6H_5	C_6H_5	$-N{=}NO^-$	Betaine		94	154-155	Orange-yellow needles	(20)
C_6H_5	C_6H_5	C_6H_5CONH-	Betaine	F	58	Decomposes	Golden yellow	(20)
C_6H_5	C_6H_5	C_6H_5CONH-	Cl		93	280.5 d.	Colorless needles	(20)
C_6H_5	C_6H_5	CN	Cl	B		265-267	Colorless needles	(182)
C_6H_5	C_6H_5	COOH	Betaine	F	90	161	Colorless rhombs	(140)
C_6H_5	C_6H_5	COOH	Cl	A		256-257 d.	Colorless needles	(140)
C_6H_5	C_6H_5	COOH	NO_3			207	Colorless tablets	(140)
C_6H_5	C_6H_5	$COOC_2H_5$	Cl	A,C	80	198-200 d.	Colorless prisms	(106, 140)†
$3\text{-}O_2NC_6H_4$	$3\text{-}O_2NC_6H_4$	$COOC_2H_5$	Cl	A		175-176	Colorless threads	(141)
$4\text{-}HOC_6H_4$	$4\text{-}HOC_6H_4$	COOH	Betaine	A	50-60	178-179	Colorless hexagonal plates	(141)‡
$4\text{-}C_2H_5OC_6H_4$	$4\text{-}C_2H_5OC_6H_4$	COOH	Cl	A	60-70	194-195	Colorless prisms	(141)
$4\text{-}C_2H_5OC_6H_4$	$4\text{-}C_2H_5OC_6H_4$	COOH	Betaine			113	Orange-yellow plates	(141)
$4\text{-}C_2H_5OC_6H_4$	$4\text{-}C_2H_5OC_6H_4$	$COOC_2H_5$	Cl	B		187	Colorless prisms	(141)
C_6H_5	C_6H_5	$COCH_3$	$PtCl_4$	B		239 d.	Yellowish	(182)
C_6H_5	C_6H_5	COC_6H_5	Cl	B		220-225	Yellowish	(182)§

* Picrate, m.p. 169°C.

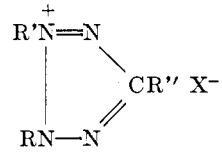
† Other salts reported.

‡ 3.5 H_2O.

§ Phenylhydrazone, m.p. 104-108°C. (d.).

TABLE 29

Tetrazolium salts with three simple aryl substituents



R	R′	R″	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	References
					per cent	°C.		
C_6H_5	C_6H_5	C_6H_5	Cl	A, C	58	243	Colorless needles	(2, 3, 70, 100, 102, 106, 121, 140, 188) (a)
C_6H_5	C_6H_5	C_6H_5	Br	D	57	255	Colorless prisms	(140)(b)
C_6H_5	4-$CH_3C_6H_4$	C_6H_5	Cl	A		229		(140, 166)
C_6H_5	4-$CH_3C_6H_4$	C_6H_5	Br	D	55	210-220	Yellowish rhombs	(2)
C_6H_5	4-iso-$C_3H_7C_6H_4$	C_6H_5	Br	D	54	186 d.	Colorless needles	(2)
C_6H_5	4-*n*-$C_{12}H_{25}C_6H_4$	C_6H_5	I	D	39	74-76	Yellow	(2)
C_6H_5	4-$C_6H_5C_6H_4$	C_6H_5	Cl	A	61-65	242-243	Orange prisms	(2, 100)
C_6H_5	C_6H_5	4-$C_6H_5C_6H_4$	Cl	A	46	220-222	Colorless needles	(89, 100)(c)
4-$C_6H_5C_6H_4$	4-$C_6H_5C_6H_4$	C_6H_5	Cl	A	29	271-273	Colorless	(100)
4-$C_6H_5C_6H_4$	4-$C_6H_5C_6H_4$	4-$C_6H_5C_6H_4$	Cl	A	75	220-221	Yellow needles	(100)(d)
C_6H_5	4-ClC_6H_4	C_6H_5	I	D		218	Yellow prisms	(2)
C_6H_5	4-IC_6H_4	C_6H_5	Cl	E	55-60	232-233		(70, 164)
C_6H_5	4-BrC_6H_4	4-BrC_6H_4	Cl	C		183-185 d.	Pale yellow	(2)
C_6H_5	2,4,6-$Br_3C_6H_2$	4-BrC_6H_4	Br	G	59	215-218 d.	Golden octahedra	(126a)
4-IC_6H_4	4-IC_6H_4	C_6H_5	Cl	E	47	256		(70)
4-$O_2NC_6H_4$	4-IC_6H_4	C_6H_5	Cl	E	20	229		(70)
C_6H_5	4-$O_2NC_6H_4$	C_6H_5	Cl	C	82	230-232 d.	Colorless needles	(2)
C_6H_5	4-$O_2NC_6H_4$	C_6H_5	Br	G	31	231-232		(108)
C_6H_5	C_6H_5	4-$O_2NC_6H_4$	Cl	D	72	250 d.	Yellow prisms	(2)
C_6H_5	4-HOC_6H_4	C_6H_5	Cl	A	78	243-244	Yellowish needles	(180)
C_6H_5	4-HOC_6H_4	4-$O_2NC_6H_4$	Cl	F	Satisfactory	208-209	Pale yellow needles	(183)
C_6H_5	4-$CH_3OC_6H_4$	C_6H_5	I	A	90	135-140	Colorless feathery crystals	(180)
C_6H_5	4-$CH_3OC_6H_4$	4-$O_2NC_6H_4$	I	B		166-168		(183)
C_6H_5	4-$CH_3OC_6H_4$	3,4-$(CH_3O)_2C_6H_3$	I	D	70	146	Lemon-yellow prisms	(2)
C_6H_5	3-$CF_3C_6H_4$	3-$CH_3OC_6H_4$	Acetate					(157)
C_6H_5	C_6H_5	4-CNC_6H_4	Cl	C	71	240 d.	Buff	(2)
3-$HOOCC_6H_4$	C_6H_5	C_6H_5	I	B	99	217	Light yellow	(191)

TABLE 29—*Continued*

R	R'	R''	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	Reference
					per cent	°C.		
C_6H_5	C_6H_5	3-$HO_3SC_6H_4$	Betaine			>250 d.	Quadrilateral tablets	(66)
C_6H_5	4-$H_2NC_6H_4$	C_6H_5	Cl	F		235-237 d.	Orange-yellow rhombs	(2)
C_6H_5	4-$CH_3CONHC_6H_4$	C_6H_5	Cl	C	42	262 d.	Yellowish prisms	(2)
C_6H_5	4-$CH_3CONHC_6H_4$	C_6H_5	I	B		289	Yellow	(187)
C_6H_5	C_6H_5	4-$H_2NC_6H_4$	Cl	F	74	285 d.	Orange	(2)
C_6H_5	C_6H_5	4-CH_3CO-NHC_6H_4	Cl	A, C	40	274 d.	Yellowish prisms	(2)
C_6H_5	C_6H_5	4-CH_3CO-NHC_6H_4	Br	G	55	276-278		(108)
C_6H_5	C_6H_5	4,4'-H_2N--C_6H_4-$SO_2NHC_6H_4$	OH	F		219	Yellow prisms or orange rhombs	(2)
C_6H_5	C_6H_5	4,4'-CH_3CONH-C_6H_4-$SO_2NHC_6H_4$	Cl	F	60	290	Yellow microcrystals	(2)
C_6H_5	2-Cl-4-$H_2NC_6H_3$	C_6H_5	Cl	F	80	186-188 d.	Orange-red prisms	(2)
C_6H_5	2-Cl-4-CH_3CONH-C_6H_5	C_6H_5	Cl	D	64	245-246 d.	Orange-red prisms	(2)
C_6H_5	3-Cl-4-$H_2NC_6H_3$	C_6H_5	Cl	D	79	250 d.	Yellow rhombs	(2)
C_6H_5	2-O_2N-4-$H_2NC_6H_3$	C_6H_5	Cl	D	19	243-245 d.	Orange-red prisms	(2)
C_6H_5	3-HO-4-$H_2NC_6H_3$	C_6H_5	Cl	A	29	210 d.	Irregular buff rhombs	(2)[e]
C_6H_5	4(n-$C_{12}H_{25}NH$)C_6H_4	C_6H_5	I	D		106-110	Reddish brown rhombs	(2)
C_6H_5	$NH_2(CH_2)_{12}NHC_6H_4$	C_6H_5	Cl	A		140-142	Pale red plates	(2)
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$	C_6H_5	Cl	A	47	110	Red powder	(126)[f]
C_6H_5	C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$	CH_3SO_4	C	41	215 d.	Pale yellow prisms	(126)[d]
C_6H_5	C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$	Cl	D	35	169-170 d.	Colorless microcrystals	(126)
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$	4-$(CH_3)_3\overset{+}{N}C_6H_4$	CH_3SO_4	C	61	244 d.	Colorless prisms	(126)[d]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$	4-ClC_6H_4	Cl	D		Decomposes	Yellow microcrystals	(126)[g]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$	4-$O_2NC_6H_4$	Cl	A	28	167-168 d.	Pale yellow microcrystals	(126)[d]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$	4-HOC_6H_4	Cl	A	52	194-195 d.	Pale yellow microcrystals	(126)[h]

TABLE 29—*Concluded*

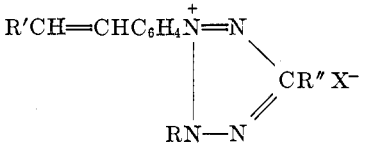
R	R'	R''	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	Reference
					per cent	°C.		
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$	4-HOC_6H_4	CH_3SO_4	C	67	240-241 d.	Pale yellow prisms	(126)[d]
4-$H_2NC_6H_4$	4-$(CH_3)_3\overset{+}{N}C_6H_4$	C_6H_5	CH_3SO_4	C	47	209 d.	Orange-red plates	(126)
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$	4-$H_2NC_6H_4$	CH_3SO_4	C	60	215-216 d.	Orange-red prisms	(126)[e]
C_6H_5	4-$H_2NC_6H_4$	4-$(CH_3)_3\overset{+}{N}C_6H_4$	Cl	A	65	175-176 d.	Buff powder	(126)[e][f]
4-$H_2NC_6H_4$	4-$(CH_3)_3\overset{+}{N}C_6H_4$	4-$H_2NC_6H_4$	CH_3SO_4	C	72	221 d.	Red prisms	(126)[j]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$	4-$HOOCC_6H_4$	CH_3SO_4	C	82	189-191 d.	White microcrystals	(126)
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$	4-$CH_3OOCC_6H_4$	CH_3SO_4	C		189-190 d.	Colorless	(126)[d]
C_6H_5	4-$(CH_3)_3\overset{+}{N}C_6H_4$	4-CNC_6H_4	Cl	G	72	decomposes at 185	Small buff prisms	(126)[d]
C_6H_5	2-HOC_6H_4	4-$(CH_3)_3\overset{+}{N}C_6H_4$	Cl	F	85	173-175	Pale yellow prisms	(126)
C_6H_5	2-$C_6H_5CH_2OC_6H_5$	4-$(CH_3)_3\overset{+}{N}C_6H_4$	Cl	G	85	158	Colorless prisms	(126)[e][d]
C_6H_5	2-ClC_6H_4	4-$(CH_3)_3\overset{+}{N}C_6H_4$	CH_3SO_4	G	52	219 d.	Colorless	(126)

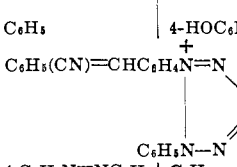
See also table 31.

[a] 0.5 HCl, 0.5 H_2O.
[b] Sesquihydrate.
[c] Monomethanolate.
[d] Monohydrate.
[e] Hydrochloride.
[f] Dihydrate.
[g] Dimethanolate.
[h] Monoethanolate.
[i] $C_8H_7OH \cdot 2H_2O$.
[j] Hemihydrate.

TABLE 30

Tetrazolium salts substituted by a styryl group

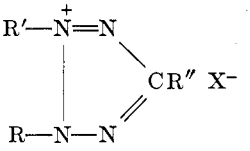


R	R'	R″	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	References
					per cent	°C.		
C_6H_5	C_6H_5	CH_3	I	D	34	169-172	Yellow needles	(127)*
C_6H_5	4-$O_2NC_6H_4$	CH_3	I	D	28	222-223	Yellow	(127)
C_6H_5	C_6H_5	C_6H_5	Cl	D	54	228 d.	Yellow needles	(127)†
C_6H_5	C_6H_5	C_6H_5	Isethionate	D	37	181	Yellow prisms	(127)
C_6H_5	C_6H_5	C_6H_5	SO_4		86	Decomposes	Yellow	(127)‡
C_6H_5	C_6H_5	C_6H_5	HSO_4		71	169-171	Yellow needles	(127)
C_6H_5	C_6H_5	4-$Br_1C_6H_4$	I	D	14	206 d.	Yellow needles	(127)
C_6H_5	C_6H_5	4-$CH_3OC_6H_4$	I	D	39	167-168 d.	Yellow needles	(127)
C_6H_5	4-BrC_6H_4	C_6H_5	Cl	D	67	216-217	Yellow needles	(127)†
C_6H_5	4-$O_2NC_6H_4$	C_6H_5	Cl	D	57	233-234	Pale yellow prisms	(127)‡
C_6H_5	4-$CH_3CONHC_6H_4$	C_6H_5	I	D	41	244 d.	Orange rectangular plates	(127)†
C_6H_5	4-HOC_6H_4	C_6H_5	I	D	25	272 d.	Orange rods	(127)
			Cl					(89, 115)
4-$C_6H_5N{=}NC_6H_4$	C_6H_5	C_6H_5	I	D	20	175 d.	Orange-red	(127)
4-$C_6H_5N{=}NC_6H_4$	C_6H_5	4-HOC_6H_4	Cl	A	58	215-216 d.	Red rods	(127)§
4-$C_6H_5N{=}NC_6H_4$	C_6H_5	4-HOOC-C_6H_4	Cl	A	11	195 d.	Red microcrystals	(127)†

* Hemihydrate. ‡ Dihydrate.
† Monohydrate. § Tetrahydrate.

TABLE 31

Tetrazolium salts substituted by a phenylazo group



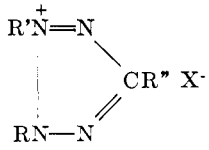
R	R′	R″	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	References
					per cent	*°C.*		
C_6H_5	4-$C_6H_5N{=}NC_6H_4$	CH_3	I	C		127-129 d.	Orange needles	(117)
C_6H_5	4-$C_6H_5N{=}NC_6H_4$	C_6H_5	I	C	24	231-232 d.	Orange	(117)
C_6H_5	C_6H_5	$C_6H_5N{=}N-$	Cl	A, C	56	249 d.	Brownish yellow prisms, violet reflex	(140, 204)[a]
C_6H_5	4-$C_6H_5N{=}NC_6H_4$	4-HOC_6H_4	Cl	A	97	267 d.	Orange-brown rhombs	(117)
4-ClC_6H_4	4-$C_6H_5N{=}NC_6H_4$	C_6H_5	I	C	33	219	Orange microcrystals	(117)
4-$HOOCC_6H_4$	4-$C_6H_5N{=}NC_6H_4$	C_6H_5	Cl	D	48	264 d.	Red prisms	(117)[b]
4-$CH_3CONHC_6H_4$	4-$C_6H_5N{=}NC_6H_4$	C_6H_5	I	C	40	258 d.	Orange fluffy needles	(117)
C_6H_5	4-4′-$CH_3C_6H_4$-$N{=}NC_6H_4$	C_6H_5	I	D	36	175-177	Ruby-red prisms	(117)
C_6H_5	4-4′-ClC_6H_4-$N{=}NC_6H_4$	C_6H_5	Cl	D	72	184-185	Red needles	(117)[c]
C_6H_5	4-4′-$O_2NC_6H_4$-$N{=}NC_6H_4$	C_6H_5	Cl	C	45	250 d.	Orange-red prisms	(117)[d]
C_6H_5	4-4′-HOC_6H_4-$N{=}NC_6H_4$	C_6H_5	Cl	A	70	230 d.	Orange-red prisms	(117)[b]
C_6H_5	4-2′-Cl-4′-HOC_6H_3-$N{=}NC_6H_4$	C_6H_5	Cl	A	26	204-205 d.	Deep red prisms	(117)[e]
C_6H_5	4-3′-Cl-4′-HOC_6H_3-$N{=}NC_6H_4$	C_6H_5	Cl	A	53	206-207 d.	Deep orange-red prisms	(117)[f]
C_6H_5	2,5-$(CH_3)_2C_6H_2$-4-$C_6H_5N{=}N$	C_6H_5	Cl	D	54	41	Orange prisms	(117)[g]
C_6H_5	α-4-$C_6H_5N{=}NC_{10}H_6$	C_6H_5	I	C	48	280 d.	Orange needles	(117)
C_6H_5	4-4′-HOC_6H_4-$N{=}NC_6H_4$	4-$(CH_3)_3\overset{+}{N}C_6H_4$	Cl	A	59	211-212 d.	Orange crystalline powder	(126)[d]

See also tables 30, 32, and 33.

[a] Thiocyanate, m.p. 160–162°C.
[b] Hemihydrate.
[c] Sesquihydrate.
[d] Dihydrate.
[e] Monohydrate.
[f] Monoethanolate.
[g] Trihydrate.

TABLE 32

Tetrazolium salts containing heterocyclic nuclei



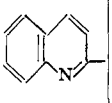
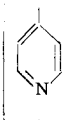
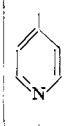
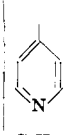
R	R'	R"	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	References
					per cent	°C.		
C_6H_5	C_6H_5		Cl	D	51	239–240	Pale yellow prisms	(34, 120, 130)
	4-ClC_6H_4		Acetate	A		205	Bright yellow needles	(154)
C_6H_5	C_6H_5		Acetate					(157)
C_6H_5	3-$CF_3C_6H_4$		Acetate	A	61	223	Pale yellow	(154)
C_6H_5	C_6H_5		Br	G		270–271		(108)
C_6H_5	C_6H_5		Cl	D	40	233 d.	Pale yellow needles	(130)*
C_6H_5	C_6H_5		Br	G	81	254-255		(108)
C_6H_5	C_6H_5		I	D	47	226-227	Orange needles	(130)
C_6H_5		C_6H_5	Cl	C	60	245 d.	Buff-yellow microcrystals	(2)
	4-ClC_6H_4	C_6H_5	Acetate	A	64	113	Yellow powder	(154)
	4-ClC_6H_4	2-ClC_6H_4	Acetate	A	43	184	Colorless	(154)

TABLE 32—*Continued*

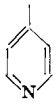
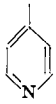
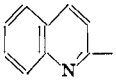
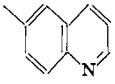
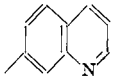
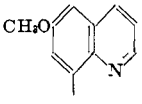
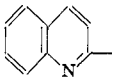
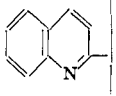
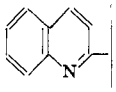
R	R′	R″	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	References
					per cent	°C.		
	4-ClC_6H_4	2-$O_2NC_6H_4$	Acetate	A	43	181	Pale yellow	(154)
	4-ClC_6H_4	2-HOC_6H_4	Acetate	A	64	248	Yellow powder	(154)
	4-ClC_6H_4	4-$CH_3OC_6H_4$	Acetate	A	86	165	Colorless	(154)
	4-ClC_6H_4		Acetate	A	34	144-146	Colorless needles	(154)
C_6H_5	4-$CH_3OC_6H_4$		Cl	D	58	157-160	Yellow needles	(130)*†
C_6H_5	C_5H_5		Br	G	65	278-280	Colorless needles	(108)
C_6H_5		C_6H_5	Picrate	A		133.5	Canary-yellow needles	(154)
C_6H_5		C_6H_5	Picrate	A		190–192	Yellow needles	(154)
C_6H_5	CH_3O 	C_6H_5	I	C	30	221	Orange plates	(2)
4-BrC_6H_4	4-BrC_6H_4		Cl	A	52‡	241	White needles	(156)
	4-ClC_6H_4	C_6H_5	Acetate	A	87	204	Yellow	(154)
	4-ClC_6H_4	2-ClC_6H_4	Acetate	A	88	220	Yellow	(154)

TABLE 32—*Continued*

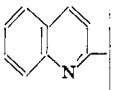
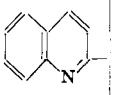
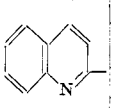
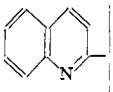
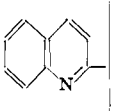
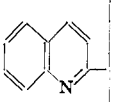
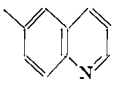
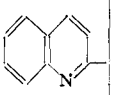
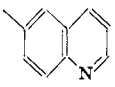
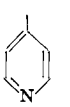
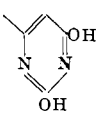
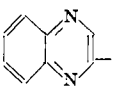
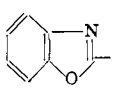
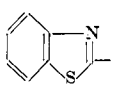
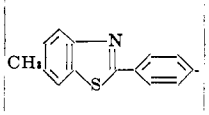
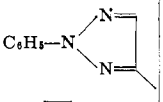
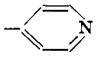
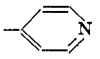
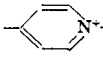
R	R′	R″	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	References
					per cent	°C.		
	4-ClC$_6$H$_4$	2-O$_2$NC$_6$H$_4$	Acetate	A	71	203	Yellow	(154)
	4-ClC$_6$H$_4$	2-HOC$_6$H$_4$	Acetate	A	44	211	Yellow	(154)
	4-ClC$_6$H$_4$	4-CH$_3$OC$_6$H$_4$	Acetate	A	87	215	Yellow powder	(154)
	4-ClC$_6$H$_4$		Acetate	A	43	223	Colorless	(154, 157)
	4-ClC$_6$H$_4$		Acetate	A	43	98	Pale yellow needles	(154)
			Acetate	A	44	204	Pale yellow	(154)
			Acetate	A		94–96	Yellow	(154)
C$_6$H$_5$	C$_6$H$_5$		Cl	A	29	224 d.	Colorless rods	(118)
4-BrC$_6$H$_4$	4-BrC$_6$H$_4$		Cl	A	65‡	229	Colorless needles	(156)
4-BrC$_6$H$_4$	4-BrC$_6$H$_4$		Cl	A		251	Colorless needles	(156)
4-BrC$_6$H$_4$	4-BrC$_6$H$_4$		Cl	A	56‡	236	White needles	(156)
C$_6$H$_5$		C$_6$H$_5$	Picrate	A		200–202	Yellow needles	(154)

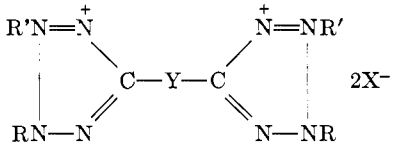
TABLE 32—*Concluded*

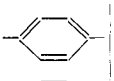
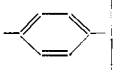
R	R′	R″	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	Reference
					per cent	°C.		
C_6H_5	C_6H_5		Cl	D	40	253-254 d.	Colorless needles	(130)
C_6H_5	4-$C_6H_5N{=}NC_6H_4$		Br	D	32	178-180	Scarlet prisms	(130)§
C_6H_5	4-$C_6H_5CH{=}CHC_6H_4$		Cl	D	51	213-216 d.	Yellow prisms	(130)§
C_6H_5	C_6H_5		HSO_4	D	5	158-160	Pale yellow prisms	(130)

* Dihydrate. † Hydrochloride. ‡ Crude yield. § Monohydrate.

TABLE 33

Tetrazolium salts containing two nuclei of the type

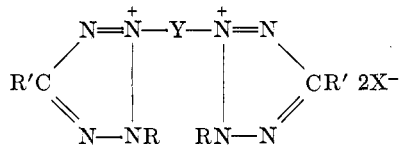


R	R′	Y	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	References
					per cent	°C.		
C_6H_5	C_6H_5		Cl	C	13	265–268		(100) [a][b]
C_6H_5	C_6H_5	$-(CH_2)_2-$	Cl	C	20	262		(100) [c][d]
C_6H_5	4-$C_6H_5N{=}NC_6H_4$	$-(CH_2)_2-$	I	C	69	185–186 d.	Dark red	(117)[e]
C_6H_5	C_6H_5	$-(CH_2)_6-$	I	D		220–224	Golden yellow rhombs	(2)[f]
C_6H_5	4-$C_6H_5N{=}NC_6H_4$	$-(CH_2)_6-$	I	D	24	169–170 d.	Red microprisms	(117)[b]
C_6H_5	C_6H_5		Cl	A	58	273-274	Colorless	(100)[g]
C_6H_5	4-$C_2H_5OOCC_6H_4$		Cl	A		322	Colorless cubes	(34, 89, 100)[c]

[a] Dihydrochloride.
[b] Monohydrate.
[c] Trihydrate.
[d] Dithiocyanate, m.p. 168°C.
[e] Monoethanolate.
[f] Tetrahydrate.
[g] Dihydrate.

TABLE 34

Tetrazolium salts containing two nuclei of the type



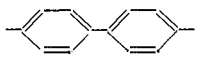
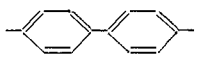
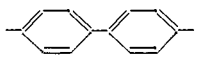
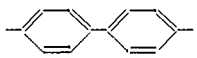
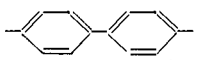
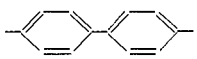
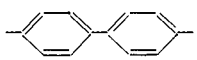
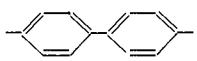
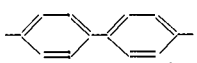
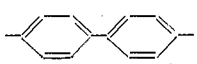
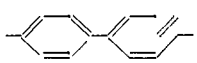
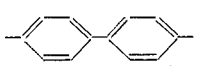
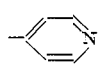
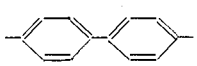
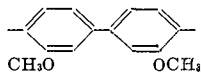
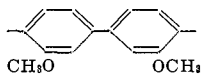
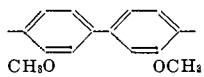
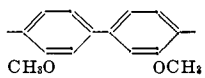
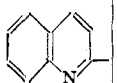
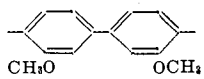
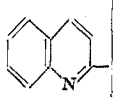
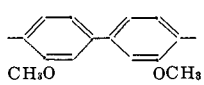
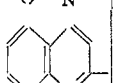
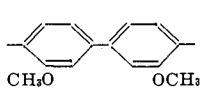
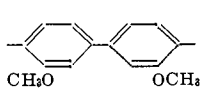
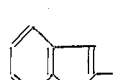
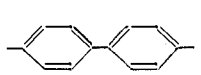
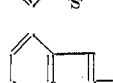
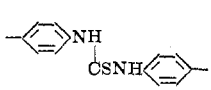
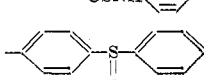
R	Y	R'	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	References
					per cent	*°C.*		
C_6H_5		CH_3	Cl	A	72	243 d.	Yellowish white	(34, 100)*
C_6H_5		C_6H_5	Cl	B,C	45	260-278 219 d.	Colorless needles	(1, 163a, 172, 186, 203)*
C_6H_5		C_6H_5	I	C	59	216.5 d.	Pale yellow	(163a)†
C_6H_5		C_6H_5	Cl	A	37-52	245-247 d.	Colorless needles	(153, 157, 160)
C_6H_5		C_6H_5	I	C	58	235 d.	Colorless needles	(163a)
C_6H_5		2-HOC_6H_4	Cl, Acetate	A	37	242	Pale yellow needles	(153, 157)
C_6H_5		4-$CH_3OC_6H_4$	Acetate	A	39	194 d.	Pale yellow needles	(153, 157)
C_6H_5		3,4-$(CH_3O)_2C_6H_3$	Acetate	A	69	194		(153, 157)
C_6H_5			Acetate	A	34	168-170	Pale yellow	(153, 154)
C_6H_5			Acetate	A	68	240 d.	Pale yellow needles	(153, 154, 157)
C_6H_5			Acetate	A		239	Colorless	(154, 157)
C_6H_5			Acetate	A	43	205 d.	Pale yellow needles	(153, 154, 157)
		C_6H_5	Acetate	A	68	223	Colorless	(154)

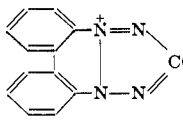
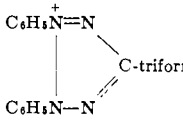
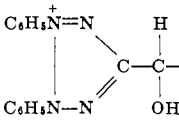
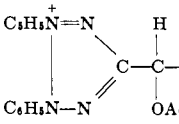
TABLE 34—*Concluded*

R	Y	R'	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	References
					per cent	°C.		
		2-ClC_6H_4	Acetate	A	43	213	Colorless	(154)
		2-$O_2NC_6H_4$	Acetate	A	34	204	Pale yellow	(154)
			Acetate	A	17	202	Yellow needles	(154)
			Acetate	A	17	173	Pale yellow needles	(154)
		C_6H_5	Acetate	A	87	223	Pale yellow	(154)
		2-ClC_6H_4	Acetate	A		182	Colorless	(154)
		2-$O_2NC_6H_4$	Acetate	A	70	174	Yellow	(154)
C_6H_5			Acetate	A	52	Decomposes at 211	Colorless needles	(153, 154, 157)
			Acetate	A	35	196-198	Yellow needles	(154, 157)
C_6H_5		C_6H_5	I	C	39	218-219		(163a)
C_6H_5		C_6H_5	I	C	36	185 d.		(163a)

* Dihydrate. † Monohydrate.

TABLE 35

Miscellaneous tetrazolium salts

Salt	Anion	Method of Synthesis	Yield	Melting Point	Appearance	References
			per cent	°C.		
	Cl			360–361	Small shining needles	(81, 107, 194)
	ClO_4			310	Needles	(81)
	Picrate			330–331	Yellow needles	(81)
	Radical		75	160–161	Olive-green needles	(107)‡
	Cl	C		Oil	Pale yellow	(79)
	Cl	F	44		Yellowish white	(205)*
	Cl	C	71	204	Colorless prisms	(205)†

* $[\alpha]_D^{20} = +21.7°$ (water). † Ac = CH_3CO. ‡ See also tables 36 and 37.

XVIII. Addendum

(February 15, 1955)

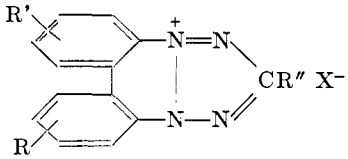
Much additional work on the chemistry of tetrazolium salts and formazans, and on their use in biological research, has been published in recent months. This addendum covers *Chemical Abstracts* to December 1953 and includes many papers published in 1954. New compounds are listed in tables 39 (formazans) and 40 (tetrazolium salts).

The earlier German work on "Photo-T.T.C." and the free radical derived therefrom has been extended (220); a number of new phototetrazolium salts have been described (table 36), their structures confirmed by reduction to the corresponding phenazones (or benzcinnolines), and free radicals (table 37) obtained by sodium dithionite reduction. A few of the tetrazolium salts used as starting materials did not give photoproducts on illumination.

Formazans and tetrazolium salts containing *N*-thiazol-2-yl groups have been described by Beyer and Pyl (209). 2-(5-Methylthiazol-2-yl)-3,5-diphenyltetrazolium bromide is so insensitive to light that a drop of an alkaline solution of this salt remains colorless when spread on filter paper in bright sunlight for

TABLE 36

Phototetrazolium salts

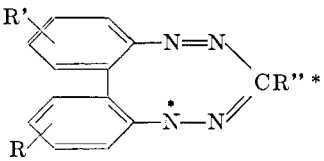
Compounds of the type 

R	R'	R"	Anion X	Yield	Melting Point	Appearance
				per cent	°C.	
—	—	C_6H_5	NO_3	80	360-361	Yellowish brown
—	—	H	NO_3	69	355	
—	—	CH_3	NO_3	42	318	Pale yellow needles
4-CH_3	4-CH_3	4-$CH_3C_6H_4$	Cl		285	*
—	4-Cl	C_6H_5	NO_3	73	291-292	Needles
—	3-Cl	C_6H_5	NO_3	85	289-291	†
—	3,4-Cl_2	C_6H_5	NO_3	40	295 d.	†
3-Cl	3-Cl	C_6H_5	NO_3	35	272 d.	†
—	3-CH_3O	C_6H_5	NO_3	40	226-230	Needles†
—	4-HOOC	C_6H_5	Br	65	374 d.	Needles
—	4-C_2H_5OOC	C_6H_5	NO_3	83	313-314	

* Sesquihydrate. † Exact orientation not established.

TABLE 37

Phototetrazolium radicals

Compounds of the type 

R	R'	R"	Yield	Melting Point	Appearance	Absorption Maximum	Susceptibility $p/10^6$
			per cent	°C.		*mμ*	
—	—	C_6H_5	75	160-161	Olive-green	500, 660	~1300
—	—	H	75	119-122	Black needles	625	1060
—	—	CH_3	92	125-127	Blue-black needles	610	~1000
4-CH_3	4-CH_3	4-$CH_3C_6H_4$			Olive-green		
—	4-Cl	C_6H_5	60	310 d.	Black needles	690	1460
—	3-Cl	C_6H_5	78	193-196	Black needles	505, 680	1090
—	3,4-Cl_2	C_6H_5	55	375	Olive-green	538, 570	950
3-Cl	3-Cl	C_6H_5	59	172-175 d.	Black needles		1090
—	3-CH_3O	C_6H_5	55	129-139	Black	650	~1000
—	4-C_2H_5OOC	C_6H_5	60	143-146	Deep blue	715	1205

* This is one of several possible conventional configurations.

many hours. The formazans have an extremely dark color (in equimolecular solution their extinction coefficients are four or five times that of triphenylformazan), and these two properties make this class of tetrazolium salts excellent as biological stains.

Kuhn and Kainer (223) have described a tetrazolyltetrazolium betaine

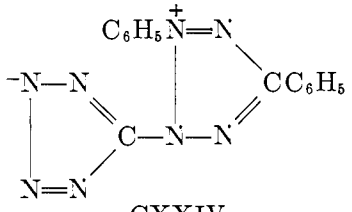
TABLE 38

Physical properties of 2,3,5-triphenyltetrazolium salts

Anion	Melting Point	Solubility in Water at 25°C.
	°C.	*grams/liter*
Iodide	228-229 d.	1.308
Triiodide	137	1.027
Tribromide	134	
Picrate	186-188*	1.037
Dichromate	218-219 d.	1.626
Perchlorate	269-270 d.	1.307
Thiocyanate	174-174.5	1.51
Ferricyanide	Explodes at 228	†

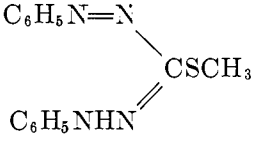
* 193-194°C. after thorough drying. † Very slightly soluble in hot water.

(CXXIV) in which the free tetrazole hydrogen is acidic enough to allow betaine formation with the quaternary tetrazolium base.

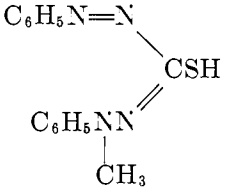


CXXIV

Further studies have been made on the tautomerism of dithizone and on its derivatives (229, 230). The *S*-methyl (CXXV) and *N*-methyl (CXXVI) derivatives of dithizone were separated by chromatography.

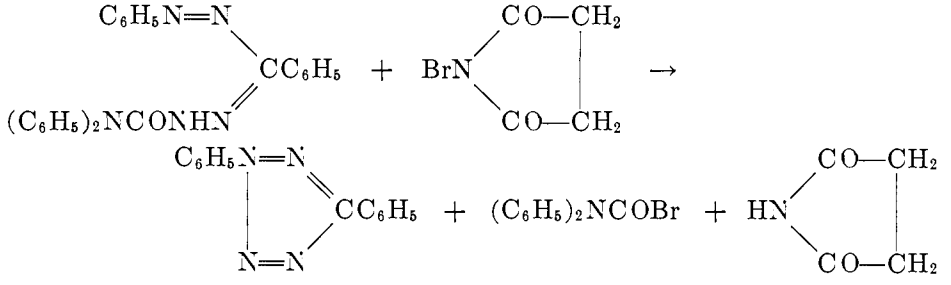


CXXV

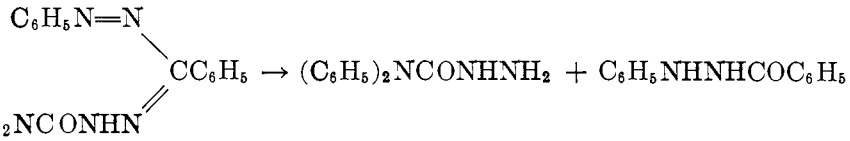


CXXVI

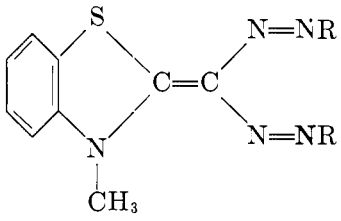
Ried and Hillenbrand (231) have investigated the azosemicarbazones further. These compounds yield no definite products on oxidation with isoamyl nitrite, lead tetraacetate, and yellow mercuric oxide, but yield tetrazoles with *N*-bromosuccinimide:



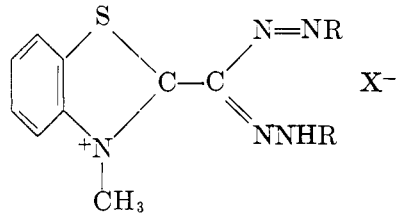
The azosemicarbazones are reduced to hydrazidines by catalytic hydrogenation, but the colorless solutions obtained become red again on exposure to air. More powerful reduction with stannous chloride splits the semicarbazone double bond:



When diazonium salts are coupled to 1,2-dimethylbenzthiazolium salts, products of the type of CXXVII are formed (236); these compounds can add on a molecule of a mineral acid to give CXXVIII, which is formally analogous to a formazan and which gives similar nickel, copper, and iron complexes. Unlike formazans, however, these substances are true bis-azo compounds in alkaline solution.



CXXVII



CXXVIII

The physical properties of a number of triphenyltetrazolium salts (table 38) are described by Weiner (240), who has also published a method for the standardization of tetrazolium salts using standard picric acid solution (239). Further polarographic studies have been reported on T.T.C. (214) and on 5-methyl-2,3-diphenyltetrazolium chloride over a wide pH range. The half-wave potential of the former at pH 7.0 is −160 mv. and of the latter −185 mv. (against a normal electrode).

In a recent patent (217) a method of producing colored photographic images on paper is described, depending on the fact that the paper is impregnated with various mixtures (e.g., ferric salts and oxalic acid) which, after exposure to light, are capable of reducing tetrazolium salts to formazans, producing a red or purple image. The tetrazolium salt may be incorporated either in the sensitized paper or in the aqueous alkaline developing solution.

It is not possible to make more than passing reference to the numerous recent publications in which tetrazolium compounds have been applied to biological problems.

Additional studies of various enzyme systems have included those on the hydrogen-transmitting respiration enzymes of *Chlorella pyrenoidosa* (242); on the enzymes in the mitochondria of rat liver and kidney (212); on a flavin enzyme extracted from *Escherichia coli* (210); on succinic dehydrogenase in the visual cells of the retina (215); and on D.P.N. and T.P.N. diaphorase in animal

TABLE 39

Addendum to tables of formazans

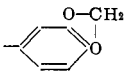
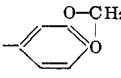
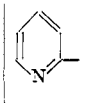
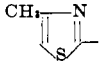
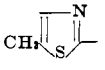
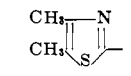
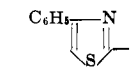
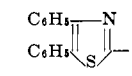
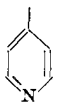
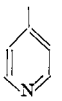
R	R′	R″	Method of Synthesis	Yield	Melting Point	Appearance	Reference
				per cent	*°C.*		
Addendum to table 12							
C_6H_5	C_6H_5	$-SCH_3$	D		116–117	Red	(229)*
	$C_6H_5N{=}N$ \ CSH // $C_6H_5N{-}N$ | CH_3		D		124	Brown	(229)†
Addendum to table 13							
2-HOC_6H_4	2-HOC_6H_4	CN	A2		195	Red, bronze reflex	(211)
Addendum to table 16							
4-$O_2NC_6H_4$	4-$O_2NC_6H_4$	C_6H_5	A1	67	222		(220)
C_6H_5	3-ClC_6H_4	C_6H_5	A1		161-162		(220)
C_6H_5	3,4-$Cl_2C_6H_3$	C_6H_5	A1		157		(220)
3-ClC_6H_4	3-ClC_6H_4	C_6H_5	A1		160		(220)
C_6H_5	2-HOC_6H_4	4-$CH_3OC_6H_4$	A1		158	Needles with golden reflex	(211)
C_6H_5	2-HOC_6H_4		A1		152	Violet-black	(211)
C_6H_5	3-$CH_3OC_6H_4$	C_6H_5	A1		131-132	Needles	(220)
4-$CH_3OC_6H_4$	4-$CH_3OC_6H_4$	4-$CH_3OC_6H_4$	A1	62	140	Needles	(220)
C_6H_5	4-$HOOCC_6H_4$	C_6H_5	A1		211	Red needles	(220)
C_6H_5	4-$C_2H_5OOCC_6H_4$	C_6H_5	A1		144	Needles	(220)
C_6H_5	2-$HOOCC_6H_4$	2-$O_2NC_6H_4$	A1		214	Red	(211)
C_6H_5	2-$HOOCC_6H_4$	4-$O_2NC_6H_4$	A1		224	Reddish brown	(211)
C_6H_5	2-$HOOCC_6H_4$	4-$CH_3OC_6H_4$	A1		173	Red	(211)
C_6H_5	2-$HOOCC_6H_4$		A1		167	Red	(211)
Addendum to table 19							
C_6H_5	2-$HOOCC_6H_4$		A1	34	202	Dark red	(234)‡
	C_6H_5	C_6H_5	A1	50	166	Black needles	(209)
	C_6H_5	C_6H_5	A1	68	160	Black needles, green reflex	(209)

TABLE 39—*Concluded*

R	R'	R''	Method of Synthesis	Yield	Melting Point	Appearance	Reference
Addendum to table 19—*Continued*							
				per cent	*°C.*		
	C_6H_5	C_6H_5	A1	69	164	Black needles, blue reflex	(209)
	C_6H_5	C_6H_5	A1	38	189-190	Black needles, green reflex	(209)
	C_6H_5	C_6H_5	A1	22	172-174	Black needles, blue reflex	(209)
Addendum to table 25							
R	R'	R''	R'''	Method of Synthesis	Yield	Melting Point	Appearance	Reference
					per cent	*°C.*		
C_6H_5	C_6H_5	C_6H_5	$3,4\text{-}(CH_3O)_2C_6H_3$	G	26	118-120	Red needles	(231)
C_6H_5	C_6H_5	C_6H_5	$2,3\text{-}(CH_3O)_2C_6H_3$	G	20	178	Red powder	(231)
C_6H_5	$\beta\text{-}C_{10}H_7$	C_6H_5	$3,4\text{-}(CH_3O)_2C_6H_3$	G	2	163-164	Deep red	(231)
C_6H_5	$\beta\text{-}C_{10}H_7$	C_6H_5	$2,3\text{-}(CH_3O)_2C_6H_3$	G	9	162	Red leaflets	(231)
C_6H_5	C_6H_5	C_6H_5		G	21	176	Dark red needles	(231)
C_6H_5	$\beta\text{-}C_{10}H_7$	C_6H_5		G	36	158	Reddish black needles	(231)
C_6H_5	$\beta\text{-}C_{10}H_7$	C_6H_5		G	32	166-167	Red	(231)
C_6H_5	$\beta\text{-}C_{10}H_7$	C_6H_5		G	1.6	168-169	Dark red	(231)
$\beta\text{-}C_{10}H_7$	$\beta\text{-}C_{10}H_7$	C_6H_5		G	Very small	179-180	Dark red	(231)

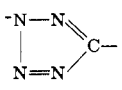
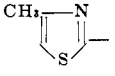
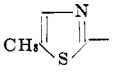
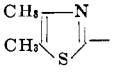
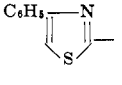
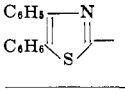
* λ_{max} absorption spectrum, 430 mμ.
† λ_{max} absorption spectrum, 545 mμ.
‡ Perchlorate, m.p. 206-207°C.

tissue (215). Detailed studies have been made of numerous factors which influence the reducing action of dehydrogenases (224, 225).

Reports have been made of the use of tetrazolium salts for staining, identifying, and counting bacteria. Neotetrazolium salt has facilitated the study of the

TABLE 40

Addendum to tables of tetrazolium salts

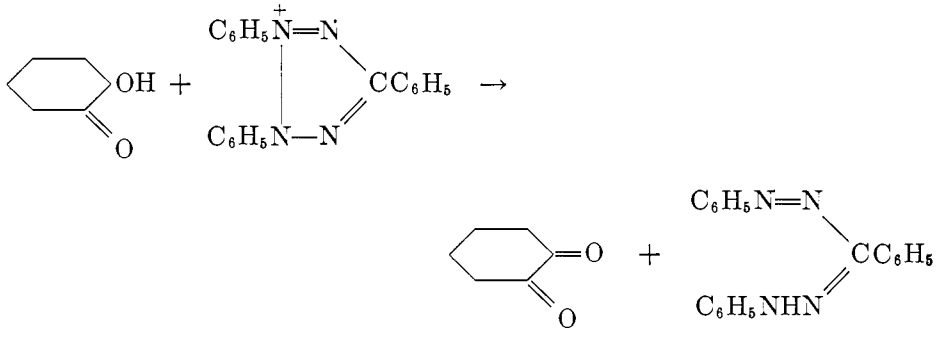
R	R′	R″	Anion X	Method of Synthesis	Yield	Melting Point	Appearance	Reference
					per cent	°C.		
Addendum to table 27								
C_6H_5	C_6H_5	$C_6H_5CH_2$	Br	G	92	194–195	Needles	(220)*
C_6H_5	C_6H_5	$C_6H_5CH_2$	Br_3	F	78	124		(220)
Addendum to table 29								
4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	Cl	A	70	221-223		(220)
C_6H_5	3-ClC_6H_4	C_6H_5	NO_3	I	90	191-192		(220)
C_6H_5	3,4-$Cl_2C_6H_3$	C_6H_5	NO_3	I	94	202-203	Needles	(220)
3-ClC_6H_4	3-ClC_6H_4	C_6H_5	NO_3	I	90	185-186	Needles	(220)
4-$O_2NC_6H_4$	4-$O_2NC_6H_4$	C_6H_5	Br	G	85	195		(220)
C_6H_5	3-$CH_3OC_6H_4$	C_6H_5	NO_3	I	90	185-187	Needles	(220)
4-$CH_3OC_6H_4$	4-$CH_3OC_6H_4$	4-$CH_3OC_6H_4$	Cl	A	82	168		(220)
C_6H_5	4-$HOOCC_6H_4$	C_6H_5	Br	G	80	241		(220)
C_6H_5	4-$C_2H_5OOCC_6H_4$	C_6H_5	NO_3	I			Colorless oil	(220)
C_6H_5	4-$C_2H_5OOCC_6H_4$	C_6H_5	Br	G			Colorless oil	(220)
Addendum to table 32								
	C_6H_5	C_6H_5	Betaine	G				(223)
	C_6H_5	C_6H_5	OH	A	74	203	Yellowish prisms	(209)†
	C_6H_5	C_6H_5	Br	G	85	149		(209)‡
	C_6H_5	C_6H_5	Br	G	80	117	Orange prisms	(209)
	C_6H_5	C_6H_5	Br	G	81	178	Pale yellow needles	(209)‡
	C_6H_5	C_6H_5	Br	G	85	171	Yellow needles	(209)
	C_6H_5	C_6H_5	Br	G		172	Orange prisms	(209)
	C_6H_5	C_6H_5	Picrate	G			Orange needles	(209)

* Monohydrate. † Picrate, m.p. 108°C. ‡ Hydrobromide.

growth of *M. tuberculosis*; it is curious that it allows this organism to grow in the presence of a concentration of streptomycin which is normally inhibitory to growth (241). Weinberg (238) has used T.T.C. in the detection and counting of bacteria and has recorded the maximum tolerated dose of T.T.C. which allows growth of Gram-positive bacteria and actinomycetes (0.001 per cent), Gram-negative bacteria (0.05 per cent), and moulds (0.25 per cent). The behavior towards T.T.C. of those bacteria found in milk has been described (233).

Weibull (237) concluded from studies on the staining of bacteria by T.T.C. that attempts to stain intracellular structures in such small organisms in this way could give misleading results. Other histochemical workers have shown that the crystalline form of the deposited formazan can obscure cell detail when certain tetrazolium salts are used as stains (216).

Reducing corticosteroids can be estimated in solution by a colorimetric estimation of the formazan produced by the reduction of compounds such as blue tetrazolium, 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride, and M & B 1767 (2,5-diphenyl-3-*p*-styrylphenyltetrazolium chloride) (214a, 218, 222). This reduction depends on the presence of an α-ketol group, and the presence of such a grouping in the alkaloid cevagenine has been demonstrated by its conversion quantitatively to dehydrocevagenine by the action of T.T.C. (235). The same reaction is described with adipoin:



The so-called "alkaline tetrazolium" reaction described by Pearse (227, 228) is an analogous procedure used for the detection (after hydrolysis) of cystine and similar compounds, and reducing sugars.

Further attempts to differentiate malignant from non-malignant tissue with the aid of tetrazolium staining have been reported as successful by some workers (232) but not by others (219).

Becker and Quadbeck (208) have shown that T.T.C. penetrates the blood–brain barrier only after death or as a result of anoxia or carbon monoxide poisoning; permeability is influenced by pH changes on either side of the barrier. A solution of T.T.C. in physiological saline injected into the renal vein of rabbits deposited formazan in the kidney; the formazan was dispersed within a few hours and was followed by epithelial necrosis (213).

Kiesewalter (221) has reviewed the uses of tetrazolium salts in medicine.

REFERENCES TO ADDENDUM

(208) BECKER, H., AND QUADBECK, G.: Z. Naturforsch. **7B**, 493 (1952).
(209) BEYER, H., AND PYL, T.: Chem. Ber. **87**, 1505 (1954).
(210) BRODIE, A. F., AND GOTS, J. S.: Science **116**, 588 (1952).
(211) CIBA LTD.: French patent 930,684 (February 2, 1948).
(212) DIANZANI, M. U.: Nature **171**, 125 (1953).
(213) DOERR, W.: Arch. pathol. Anat. u. Physiol. (Virchow's) **321**, 537 (1952); Chem. Abstracts **47**, 769i (1953).
(214) DOSKOČIL, J.: Sborník Mezinárod. Polarog. Sjezdu Praze, 1st Congr. **1951**, Pt. III, Proc., 649; Chem. Abstracts **47**, 11033g (1953).
(214a) ELLIOTT, F. H., BIRMINGHAM, M. K., SCHALLY, A. V., AND SCHONBAUM, E.: Endocrinology **55**, 721 (1954).
(215) FARBER, E., STERNBERG, W. H., AND DUNLOP, C. E.: Proc. Soc. Exptl. Biol. Med. **86**, 534 (1954).
(216) FRANCIS, C. M.: J. Physiol. **119**, (4), 38 P (1952).
(217) GEVAERT LTD.: British patent 670,883 (April 30, 1952).
(218) HENLY, A. A.: Nature **169**, 877 (1952).
(219) HSU, Y. T., AND HOCH-LIGETI, C.: Am. J. Pathol. **29**, 105 (1953).
(220) JERCHEL, D., AND FISCHER, H.: Ann. **590**, 216 (1954).
(221) KIESEWALTER, J.: Pharmazie **7**, 380 (1952).
(222) KIRSTEN, W., AND SJÖSTEDT, U.: Mikrochemie ver. Mikrochim. Acta **1954**, 730.
(223) KUHN, R., AND KAINER, H.: Angew. Chem. **65**, 442 (1953).
(224) KUHN, R., AND LINKE, F.: Ann. **578**, 155 (1952).
(225) KUHN, R., PFLEIDERER, G., AND SCHULZ, W.: Ann. **578**, 159 (1952).
(226) LETTRÉ, H., HAEDE, W., AND SCHÄFER, L.: Hoppe-Zeyler's Z. physiol. Chem. **289**, 298 (1952).
(227) PEARSE, A. G. E.: J. Histochem. Cytochem. **1**, 460 (1953).
(228) PEARSE, A. G. E.: J. Pathol. Bacteriol. **67**, 129 (1954).
(229) PEL'KIS, P. S.: Doklady Akad. Nauk S.S.S.R. **88**, 999 (1953); Chem. Abstracts **48**, 9943 (1954).
(230) PUPKO, L. S., AND PEL'KIS, P. S.: Zhur. Obshcheĭ Khim. **24**, 1640 (1954).
(231) REID, W., AND HILLENBRAND, H.: Ann. **590**, 128 (1954).
(232) SCHÜMMELFEDER, N., AND SCHÜMMELFEDER, W.: Z. Krebsforsch. **59**, 223 (1953).
(233) SCHWARZ, G., CIBLIS, E., AND LANGE, W.: Milchwissenschaft **8**, 261 (1953); Chem. Abstracts **47**, 12676i (1953).
(234) SEYHAN, M.: Chem. Ber. **87**, 1124 (1954).
(235) STOLL, A., STAUFFACHER, D., AND SEEBECK, E.: Helv. Chim. Acta **36**, 2027 (1953).
(236) WAHL, H., AND LE BRIS, M-TH.: Bull. soc. chim. France **1954**, 248.
(237) WEIBULL, C.: J. Bact. **66**, 137 (1953).
(238) WEINBERG, E. D.: J. Bact. **66**, 240 (1953).
(239) WEINER, S.: Chemist Analyst **42**, 9 (1953).
(240) WEINER, S.: J. Am. Chem. Soc. **76**, 5814 (1954).
(241) WINTERSCHEID, L. C., GLICK, M. C., AND MUDD, S.: Am. Rev. Tuberc. **68**, 625 (1953).
(242) ZIEGLER, H.: Naturwissenschaften **40**, 144 (1953).