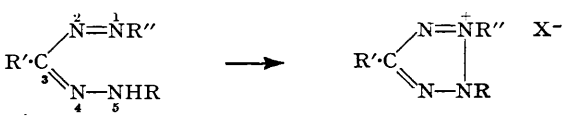
793. *Tetrazolium Compounds. Part I. Tetrazolium Compounds containing Substituted Phenyl and Heterocyclic Rings.*

By J. N. Ashley, (Miss) B. M. Davis, A. W. Nineham, and R. Slack.

Substituted derivatives of 2 : 3 : 5-triphenyltetrazolium chloride have been prepared in order to study their biological potentialities. Particular attention has been paid to the introduction of amino- and substituted amino-groups. Two compounds containing heterocyclic groups and one bistetrazolium salt are also described. Various methods of oxidising formazans to tetrazolium salts have been examined.

During the last few years a number of substituted triphenyltetrazolium salts has been prepared for various biological purposes, *e.g.*, tissue staining and the demonstration of reducing enzymes in normal and neoplastic tissues. Smith (*Science*, 1951, **113**, 753) has reviewed some of the uses of a few well-known compounds.

The only general method of preparing tetrazolium salts is by the oxidation of formazans : *

(I) 

The oxidising agents usually employed are nitrous acid, *iso*amyl nitrite, mercuric oxide, or lead tetra-acetate (Kuhn and Jerchel, *Ber.*, 1941, **74**, 941). There appears to be no ready

* Various methods of naming compounds of type (I) have been used. In agreement with the Editor, the nomenclature in this and subsequent papers of the series will be based on the Beilstein name " formazan " for the hypothetical parent compound (I; R = R′ = R″ = H), the numbering being as shown. The compound (I; R′ = H, R = R″ = Ph), often called simply " formazyl," becomes 1 : 5-diphenylformazan.

way of predicting which of these will be suitable and the final choice is usually made by trial. There are distinct resemblances between the oxidation–reduction system, formazan $\underset{2H}{\overset{O}{\rightleftharpoons}}$ tetrazolium salt, and the well-known thermodynamically reversible systems, *e.g.*, quinones $\rightleftharpoons$ quinols, and azo-compounds $\rightleftharpoons$ hydrazo-compounds. Some such processes, involving the overall reaction of two electrons per molecule, proceed by stepwise addition of individual electrons (cf. Michaelis and Schubert, *Chem. Reviews*, 1938, **22**, 437) and it is possible that the tetrazolium salt–formazan relationship might show similar characteristics. However, little is known about the actual mode of reduction. At the mercury electrode, reduction apparently proceeds by a *four*-electron process to a stage beyond the formazan (Campbell and Kane, personal communication) * and it is by no means certain that the formazan is formed even as an intermediate. The oxidising agents which are effective in the reverse reaction are mainly characteristic dehydrogenators. Indeed, it has been noted during this work that some formazans appear to undergo a slow, but definite, autoxidation. It has also been shown that, if the oxidation of 1 : 3 : 5-triphenylformazan to a 2 : 3 : 5-triphenyltetrazolium salt is effected at room temperature with lead tetra-acetate in a closed system connected by a differential manometer, there is a very marked uptake of oxygen by the reacting formazan. Hence, under such conditions, some species capable of rapid reaction with atmospheric oxygen is formed transiently, and the most likely possibility is an odd-electron intermediate of the type R–$\dot{\underset{\cdot\cdot}{N}}$–N=CR′–N=$\underset{\cdot\cdot}{N}$–R. This hypothesis should be capable of rigorous physicochemical proof and further work is being done † Meanwhile, an alternative oxidation procedure has provided a measure of corroborative evidence. Although 1 : 3 : 5-triphenylformazan in dilute alcohol is attacked very slowly by hydrogen peroxide, addition of a trace of ferrous iron causes immediate oxidation of dissolved formazan. This may be an example of the reactivity of the free hydroxy radical (cf. Haber and Weiss, *Proc. Roy. Soc.*, 1939, *A*, **147**, 332), or of some secondary organic radical, *e.g.*, $CH_3 \cdot CH(OH) \cdot$ produced from the alcoholic substrate (cf. Mackinnon and Waters, *J.*, 1953, 323). Ferric iron itself will not perform the oxidation.

Two other methods of oxidation have been examined. Aqueous sodium hypochlorite converted 1 : 3 : 5-triphenylformazan in acetic acid smoothly into 2 : 3 : 5-triphenyltetrazolium chlorate, some inorganic hypochlorite decomposing at the temperature of the reaction to give the chlorate ion. Potassium chlorate itself is ineffective. The chlorate ion of the quaternary salt was reduced to chloride by ferrous iron, the tetrazolium nucleus being unaffected. Chromium trioxide in acetic acid oxidised the formazan to 2 : 3 : 5-triphenyltetrazolium dichromate. It is not suggested that these methods of oxidation show any advantage over the established procedures which have been used in this work.

The formazans (Table 1) required for the synthesis of the various tetrazolium salts described were prepared by the condensation of appropriate diazonium chlorides with phenylhydrazones in the presence of pyridine. The following formazans did not appear to be formed under these conditions: 5-*p*-dimethylaminophenyl-1 : 3-diphenyl-, 3-*p*-dimethylaminophenyl-1 : 5-diphenyl-, 1 : 5-diphenyl-3-styryl-, 3-phenyl-1-2′-pyridyl-5-3′-pyridyl-, and 5-(4-acetamido-3-hydroxyphenyl)-1 : 3-diphenyl-formazan. In cases where one nucleus of the phenylhydrazone carried an activating substituent such as dimethylamino or hydroxyl, nuclear coupling may have occurred preferentially (cf. Hausser, Jerchel, and Kuhn, *Ber.*, 1951, **84**, 651) for, when hydroxyl groups were acetylated before coupling, formazan formation proceeded smoothly. In certain other cases, notably where long-chain substituents were concerned, the formazans were formed but could not be obtained solid or analytically pure. Examples studied were octamethylenebis-3-(1 : 5-diphenylformazan), 5-*p*-hexylphenyl-1 : 3-diphenyl-, 5-(4-*N*-acetyldodecylaminophenyl)-1 : 3-diphenyl-, and 5-(*N*-12′-acetamidododecyl-*N*-acetyl-4-aminophenyl)-1 : 3-diphenyl-formazan. The last two were oxidised to tetrazolium salts which were successfully purified. Again, 3-*p*-bromophenyl-1-phenyl-5-(2 : 4 : 6-tribromophenyl)-, 3 : 5-di-*p*-cyanophenyl-1-

* A polarographic examination has also been reported by Ried and Wilk (*Annalen*, 1953, **581**, 49).

† Kuhn and Jerchel (*Annalen*, 1952, **578**, 1) recently prepared the stable, crystalline, 2 : 3-diphenylene-5-phenyltetrazolium radical by cautious reduction of the corresponding salt, an experiment which supports the general hypothesis of radical intermediates.

phenyl-, 5-*p*-(2-diethylaminoethoxycarbonyl)phenyl-1 : 3-diphenyl-, and 1 : 3-diphenyl-5-*p*-*N*-(4-diethylamino-1-methylbutyl)sulphamylphenyl-formazan could not be oxidised satisfactorily.

Most of the phenylhydrazones mentioned were known, but some of the amines used for diazotisation were new and were obtained from the appropriate acetanilides by nitration

TABLE 1. *Formazans*, Ph·NH·N:CR′·N:NR″.

No.	R′	R″	Yield (%)	M. p.	Appearance	Solvent
1 [a]	*p*-C_6H_4·NO_2	Ph	40	204°	Dark-red needles, green reflex	$COMe_2$
2 [b]	Ph	*p*-C_6H_4·NO_2	92	195	Greenish-black needles	Aq. $COMe_2$
3 [c]	Ph	*p*-C_6H_4Cl	60	117	—	,,
4 [d]	Ph	*p*-$C_6H_4Pr^i$	—	113—114	Red prisms	MeOH
5	*p*-C_6H_4Br	*p*-C_6H_4Br	80	203	Black needles	$CHCl_3$–EtOH
6 *	,,	2 : 4 : 6-$C_6H_2Br_3$	10	169	Black needles, purple reflex	,,
7 [e]	3 : 4-$(MeO)_2C_6H_3$	*p*-C_6H_4·OMe	25	152—153	Reddish-brown needles	Aq. $COMe_2$
8	*p*-C_6H_4·CN	Ph	64·5	215	Red needles, metallic reflex	$COMe_2$
9	,,	*p*-C_6H_4·CN	80	229 †	Purple needles, green reflex	$PhNO_2$
10 [f]	Ph	*p*-C_6H_4Ph	44	176	Dark-red	Aq. $COMe_2$
11	Ph	3-Pyridyl	53	168—169	Purple prisms, yellow reflex	,,
12	Ph	8-6′-Methoxyquinolyl	20	178—179	—	,,
13	Ph	*p*-C_6H_4·$C_{12}H_{25}$	83	85	Red needles, green reflex	EtOH
14 [g]	–$[CH_2]_6$–	Ph	—	129—132	Dark-red powder	*cyclo*Hexane
15 [h]	*p*-C_6H_4·NHAc	Ph	53	206—207	—	Pyridine
16	,,	*p*-C_6H_4·NHAc	17	236—237	—	Aq. $COMe_2$
17	Ph	2 : 4-C_6H_3Cl·NHAc	76	184—185	Black prisms, red reflex	EtOH
18	Ph	3 : 4-C_6H_3Cl·NHAc	44	184—185	Purple needles, yellow reflex	EtOAc
19	Ph	2 : 4-$C_6H_3(NO_2)$·NHAc	57	210·5—212	Brownish-purple plates	,,
20	Ph	3 : 4-$C_6H_3(OAc)$·NHAc	39	217—218	Reddish-purple needles	$COMe_2$
21	Ph	*p*-C_6H_4·NAc·$C_{12}H_{25}$	—	—	Red oil	—
22	Ph	*p*-C_6H_4·NAc·$[CH_2]_{12}$·NHAc	—	—	Red gum	—
23 [a]	Ph	*p*-C_6H_4·CO·O·$[CH_2]_2$·NEt_2,HCl	64	172	Dark red prisms, coppery reflex	EtOH
24	Ph	*p*-C_6H_4·SO_2·NH·CHMe·$[CH_2]_3$·NEt_2,HCl	47	194 †	Red prisms	,,

[a] Prepared by D. D. Libman. [b] Mentioned by Wedekind and Stauwe (*Ber.*, 1898, **31**, 1756) as of m. p. 165—170°, and by Busch and Schmidt (*J. pr. Chem.*, 1931, **131**, 182) but not described. [c] Described by Hausser, Jerchel, and Kuhn (*Ber.*, 1949, **82**, 515) as deep red needles, m. p. 164—165°. [d] Wedekind and Stauwe (*loc. cit.*) give m. p. 173—174° but do not describe it. [e] Prepared by D. W. Mathieson. [f] Wedekind (*Annalen*, 1898, **300**, 239) gives m. p. 174°; Jerchel and Fischer (*Annalen*, 1949, **563**, 200) give m. p. 167—168°. [g] Prepared by D. L. Pain. This is the bisformazan. [h] Prepared by A. L. Tarnoky.

* Could not be oxidised. † With decomp.

and reduction. 2-Acetamido-5-aminophenyl acetate was obtained most satisfactorily from 2-methyl-1 : 3-benzoxazole (Hewitt and King, *J.*, 1926, 822). In our hands, nitration gave, not 2-methyl-6-nitro-1 : 3-benzoxazole as claimed by Newbery and Phillips (*J.*, 1928, 122), but 2-acetamido-5-nitrophenol, which was acetylated and reduced to 2-acetamido-5-aminophenyl acetate (cf. Phillips, *J.*, 1930, 2685).

Throughout the work, recrystallisation of the tetrazolium salts to a state of analytical purity has been difficult, particularly with increasing complexity of the molecule. Many of the poor yields quoted in Table 2 are attributed to losses during recrystallisation. Salts often retained solvent of crystallisation very tenaciously and crystalline form varied widely with the conditions of crystallisation. Melting points are not characteristic, being usually accompanied by decomposition.

Experimental

p-*Cyanobenzaldehyde phenylhydrazone*, prepared (52%) in boiling ethanol, separated in flattened yellow needles, m. p. 148—149°, from aqueous acetic acid (1 : 4) (Found: N, 19·1. $C_{14}H_{11}N_3$ requires N, 19·0%).

p-*Cyanobenzaldehyde* p-*cyanophenylhydrazone* was obtained (96%) as pale yellow needles, m. p. 246° (from ethanol) (Found: N, 22·9. $C_{15}H_{10}N_4$ requires N, 22·7%). With *p*-cyanobenzenediazonium chloride in pyridine it gave mainly 4 : 4′-dicyanobenzophenone *p*-cyanophenylhydrazone.

2-Chloro-4-nitroacetanilide, prepared according to Chattaway *et al.* (*Ber.*, 1900, **33**, 3057) and crystallised from methanol, gave cream-coloured needles (71%), m. p. 131—134° (lit., 143°), and, from carbon tetrachloride, colourless needles, m. p. 169—171° (Found: Cl, 16·5. Calc. for $C_8H_7O_3N_2Cl$: Cl, 16·5%).

4-*Amino*-3-*chloroacetanilide*.—3-Chloro-4-nitroacetanilide (Fourneau, Trefouel, and Wancolle, *Bull. Soc. chim.*, 1930, **47**, 738) (40 g.) in boiling glacial acetic acid (65 c.c.) and water (80 c.c.) was treated with iron dust (30 g.) at a rate sufficient to maintain gentle boiling. The whole was then heated under reflux for 15 min. and filtered while hot. Basification of the filtrate gave the *anilide* as needles (57%), m. p. 115—116° (from benzene–light petroleum) (Found: N, 15·4; Cl, 19·3. $C_8H_9ON_2Cl$ requires N, 15·2; Cl, 19·3%).

2-*Acetamido*-5-*aminophenyl Acetate*.—2-Acetamido-5-nitrophenyl acetate (74% yield from the phenol) was hydrogenated in ethanol in the presence of Adams's platinum oxide at room temperature and pressure. The product crystallised from ethanol as the *monoalcoholate* (91%), colourless prisms, m. p. 210—211° (Found: N, 11·7. $C_{10}H_{12}O_3N_2,C_2H_6O$ requires N, 11·0%).

5-*Acetamido*-2-*aminophenyl acetate* was obtained by similar hydrogenation of a suspension of the nitro-compound in glacial acetic acid and, crystallised from methanol, had m. p. 240—245° (decomp.) (Found: C, 57·8; H, 5·8; N, 13·3. $C_{10}H_{12}O_3N_2$ requires C, 57·7; H, 5·8; N, 13·4%).

1-p-*Nitroanilinododecane*.—*p*-Nitroaniline (14 g.), dodecyl iodide (30 g.), potassium carbonate (14 g.), and copper bronze (0·5 g.) were heated at 160—180° (bath-temp.) for 4 hr. The cooled melt was extracted with boiling water, dried, and extracted with cold light petroleum. The insoluble residue was extracted with hot benzene, the extract was dried ($MgSO_4$), the solvent removed, and the residue distilled, giving a bright yellow waxy solid, b. p. 222—224°/0·05 mm. (33%). This was recrystallised from methanol to give the *base* as yellow prisms, m. p. 64—65° (Found: C, 70·7; H, 10·1; N, 9·2. $C_{18}H_{30}O_2N_2$ requires C, 70·6; H, 9·8; N, 9·1%).

With acetic anhydride and a drop of concentrated sulphuric acid it gave the *acetyl* derivative, needles, m. p. 60—60·5°, from a large volume of aqueous methanol (1 : 9) (Found: N, 8·0. $C_{20}H_{32}O_3N_2$ requires N, 8·05%).

1-N-p-*Aminophenylacetamidododecane*.—The nitro-compound (19 g.), suspended in methanol (150 c.c.), was hydrogenated in the presence of Adams's platinum oxide (0·5 g.) at room temperature and pressure (hydrogen uptake 82·5%). The solution was filtered, the solvent was removed *in vacuo*, the oily residue was dissolved in ether, and the solution treated with dry hydrogen chloride. The ether was decanted and the product was dissolved in acetone. Dry ether was added until crystallisation began. Recrystallisation from dry acetone gave the *hydrochloride* as nacreous plates (64%), m. p. 165—168° (Found: N, 7·6; Cl, 9·6. $C_{20}H_{35}ON_2Cl,H_2O$ requires N, 7·5; Cl, 9·5%).

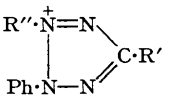
1-p-*Methoxyphenoxy*-12-p-*nitroanilinododecane*.—A mixture of 1-iodo-12-*p*-methoxyphenoxydodecane (70 g.) (Ziegler, Weber, and Gellert, *Ber.*, 1942, **75**, 1715), *p*-nitroaniline (64 g.), and powdered anhydrous potassium carbonate (25 g.) was heated at 150—160° (internal; critical) for 3½ hr. The mass was cooled and extracted with boiling chloroform, and the solution was filtered and evaporated. The residue crystallised from ethanol (charcoal) to give 1-p-*methoxyphenoxy*-12-*p*-*nitroanilinododecane* (55 g.), lemon-yellow leaflets, m. p. 84—85° (Found: C, 70·5; H, 8·7; N, 5·6, 5·4, 5·6. $C_{25}H_{36}O_4N_2$ requires C, 70·1; H, 8·4; N, 6·5%). Correct N (Dumas) values could not be obtained. Yields varied from 52 to 82% and diminished with increasing scale. They were not improved by the use of copper bronze or cuprous iodide.

On use of equimolar quantities of reactants, only NN-*di*-(12-p-*methoxyphenoxydodecyl*)-p-*nitroaniline*, pale yellow fluffy needles [from light petroleum (b.p. 60—80°)], m.p. 102—103°, was isolated (Found: N, 3·7. $C_{44}H_{66}O_6N_2$ requires N, 3·7%).

1-*Bromo*-12-p-*nitroanilinododecane*.—1-*p*-Methoxyphenoxy-12-*p*-nitroanilinododecane (55 g.) was mixed with acetic anhydride (180 c.c.), and 60% hydrobromic acid (180 c.c.) was added dropwise with stirring. The mixture was heated under reflux with stirring for 2 hr. and poured into water. The resulting solid was filtered off, washed with water, and dissolved in hot benzene.

Water was removed azeotropically and the solution was filtered (charcoal). The yellow crystalline *bromide* (40%), m. p. 63—64°, was obtained by the cautious addition of light petroleum (b. p. 60—80°) (Found: N, 7·6; Br, 20·7. $C_{18}H_{29}O_2N_2Br$ requires N, 7·3; Br, 20·8%). Conditions were critical. Yields varied from 33 to 55% and decreased with longer reaction times; no desired product was obtained after 24 hours' reaction or in a sealed tube at 120°. Only 1 : 12-di-iodododecane was isolated when hydriodic acid was used.

TABLE 2. *Tetrazolium salts,*



No.	R′	R″	Anion	Method of oxidn.	Yield (%)	M. p.	Appearance	Solvent *
1 [a]	*p*-C_6H_4·NO_2	Ph	Cl	A	72	250° †	Pale yellow prisms	$CHCl_3$ or aq. HCl
2 [b]	Ph	*p*-C_6H_4·NO_2	Cl	B	82	230—232 †	Colourless needles	$COMe_2$
3	Ph	*p*-C_6H_4Cl	I	A	—	218	Yellow prisms	EtOH
4	Ph	*p*-$C_6H_4Pr^i$	Br	A	54	186 †	Colourless needles	EtOH–Et_2O
5	*p*-C_6H_4Br	*p*-C_6H_4Br	Cl	B	—	183—185 †	Pale yellow	MeOH
6	*p*-C_6H_4·CN	Ph	Cl	B	71	240 †	Buff	EtOH–Et_2O
7	3 : 4-$C_6H_3(OMe)_2$	*p*-C_6H_4·OMe	I	A	70	146	Lemon-yellow prisms	50% EtOH
8 [c]	Ph	*p*-C_6H_4Ph	Cl	A	65	238 †	Orange prisms	$CHCl_3$–Et_2O
9	Ph	3-Pyridyl	Cl	B	60	245 †	Buff-yellow	EtOH–Et_2O
10	Ph	8-6′-Methoxyquinolyl	I	B	30	221	Orange plates	EtOH
11	Ph	*p*-C_6H_4·$C_{12}H_{25}$	I	A	39	74—76	Yellow	$CHCl_3$–Pet
12 [d]	Ph	*p*-C_6H_4Me	Br	A	55	210—220	Pale yellow rhombs	—
13 [e]	–$[CH_2]_6$–	Ph	I	A	—	220—224	Golden-yellow rhombs	EtOH–Pet
14 [f]	Ph	*p*-C_6H_4·NHAc	Cl	B	42	262 †	Pale yellow prisms	MeOH–Et_2O
15	Ph	*p*-C_6H_4·NH_2	Cl	—	—	235—237 †	Orange-yellow rhombs	,,
16 [b, g]	*p*-C_6H_4·NHAc	Ph	Cl	B, C	40	274 †	Pale yellow prisms	H_2O
17	*p*-C_6H_4·NH_2	Ph	Cl	—	74	285 †	Orange	EtOH
18	*p*-(*p*-Acetamidophenylsulphonamido)phenyl	Ph	Cl	—	60	290	Yellow	75% EtOH
19	*p*-(*p*-Aminophenylsulphonamido)phenyl	Ph	OH	—	—	219	Yellow elongated prisms or orange rhombs	Aq. pyridine (1 : 1)
20	Ph	2 : 4-C_6H_3Cl·NHAc	Cl	A	64	245—246 †	Orange-red prisms	EtOH–Et_2O
21	Ph	2 : 4-C_6H_3Cl·NH_2	Cl	—	80	186—188 †	Orange-red prisms	,,
22	Ph	3 : 4-C_6H_3Cl·NH_2	Cl	A	79	250 †	Yellow rhombs	,,
23	Ph	2 : 4-$C_6H_3(NO_2)$·NH_2	Cl	A	19	243—245 †	Orange-red prisms	,,
24	Ph	3 : 4-$C_6H_3(OH)$·NH_2,HCl	Cl	C	29	210 †	Buff rhombs	,,
25	Ph	*p*-C_6H_4·NH·$C_{12}H_{25}$	I	A	—	106—110	Reddish-brown rhombs	C_6H_6–Pet
26	Ph	*p*-C_6H_4·NH·$[CH_2]_{12}$·NH_2,HCl	Cl	C	—	140—142	Pale red plates	$COMe_2$ or H_2O

Methods of oxidation: A, Mercuric oxide; B, lead tetra-acetate; C, *iso*amyl nitrite.

[a] Prepared by D. D. Libman. [b] Bromide prepared by Kuhn and Münzing, *Chem. Ber.*, 1953, **86**, 858. [c] Jerchel and Fischer (*Annalen*, 1949, **563**, 200) give m. p. 242—243°. [d] von Pechmann (*Ber.*, 1894, **27**, 2930) prepared the chloride, m. p. 229°. [e] Prepared by D. L. Pain. This is the bis-compound. [f] Wedekind (*Ber.*, 1899, **32**, 1919) prepared the iodide, m. p. 289°. The bromide, m. p. 287° (from water), was also prepared during the present work. [g] Prepared by A. L. Tarnoky.

* Pet. = light petroleum (b. p. 60—80°). † With decomp.

1-p-*Nitroanilino*-12-*phthalimidododecane*.—1-Bromo-12-*p*-nitroanilinododecane (35 g.) and potassium phthalimide (19 g.) were heated in an oil-bath at 130—150° for 2½ hr. The cooled melt was extracted with boiling alcohol, and the solution was filtered (charcoal). The *imide* separated as yellow needles (97%), m. p. 116° (Found : C, 68·8; H, 7·3; N, 9·3. $C_{26}H_{33}O_4N_3$ requires C, 69·2; H, 7·3; N, 9·3%).

1-*Amino*-12-p-*nitroanilinododecane*.—The foregoing imide (11·2 g.) was heated under reflux in methanol (100 c.c.) and chloroform (35 c.c.) with hydrazine hydrate (50%; 7 c.c.) for 8 hr. The mixture was evaporated almost to dryness and the residue stirred with dilute aqueous ammonia. The yellow product was filtered off, washed with water, and crystallised from methanol, to give yellow irregular prisms (94%), m. p. 86—87° (Found : N, 12·8. $C_{18}H_{31}O_2N_3$ requires N, 13·1%). Attempts to prepare this *diamine* through the hexamethylenetetramine adduct gave only intractable tars.

Acetylation with acetic anhydride and a few drops of glacial acetic acid gave, after recrystallisation of the product from methanol (charcoal), N-p-*nitrophenyl*-1 : 12-*diacetamidododecane* (91%), yellow rectangular plates, m. p. 83° (Found : N, 10·7. $C_{22}H_{35}O_4N_3$ requires N, 10·4%).

N-p-*Aminophenyl*-1 : 12-*diacetamidododecane*.—The diacetyl compound (25 g.) was hydrogenated in methanol solution (120 c.c.) at room temperature and pressure in the presence of Adams's platinum oxide (0·5 g.) (95% uptake). The solution was filtered and the filtrate was saturated with dry hydrogen chloride. The thick oil remaining after evaporation of the solvent was used directly for diazotisation. The bright yellow *picrate*, m. p. 100°, separated from ethyl acetate (Found : N, 12·5. $C_{28}H_{40}O_9N_6,C_4H_8O_2$ requires N, 12·1%).

TABLE 3. *Analyses of formazans.*

		Found (%)				Required (%)			
No.*	Formula	C	H	N	Hal.	C	H	N	Hal.
1	$C_{19}H_{15}O_2N_5$	66·3	4·2	21·0	—	66·1	4·4	20·3	—
2	$C_{19}H_{15}O_2N_5$	66·4	4·4	20·3	—	66·1	4·4	20·3	—
3	$C_{19}H_{15}N_4Cl$	68·1	4·6	16·7	10·8	68·2	4·5	16·7	10·6
4	$C_{32}H_{22}N_4$	77·2	6·6	16·5	—	77·2	6·4	16·4	—
5	$C_{19}H_{14}N_4Br_2$	49·8	3·1	12·0	34·9	49·8	3·1	12·2	34·9
6	$C_{19}H_{12}N_4Br_4$	37·2	1·9	9·3	51·5	37·0	1·9	9·1	51·9
7	$C_{22}H_{22}O_3N_4$ [a]	67·2	5·9	14·0	—	67·7	5·7	14·3	—
8	$C_{20}H_{15}N_5$	—	—	22·0	—	—	—	21·6	—
9	$C_{21}H_{14}N_6$	72·0	4·0	24·0	—	71·5	4·1	24·1	—
10	$C_{25}H_{20}N_4$	79·8	5·6	14·9	—	79·8	5·3	14·9	—
11	$C_{18}H_{15}N_5$	71·8	5·3	22·8	—	71·8	5·0	23·3	—
12	$C_{23}H_{19}ON_5$ [b]	72·2	5·0	18·6	—	72·5	5·0	18·4	—
13	$C_{31}H_{40}N_4$	79·1	8·6	11·8	—	79·3	8·7	11·9	—
14	$C_{32}H_{34}N_8$	72·5	6·4	20·5	—	72·4	6·4	21·2	—
15	$C_{21}H_{19}ON_5$	—	—	18·9	—	—	—	19·6	—
16	$C_{23}H_{22}O_2N_6$	65·7	5·5	19·9	—	66·6	5·5	20·2	—
17	$C_{21}H_{18}ON_5Cl$	—	—	—	9·2	—	—	—	9·1
18	$C_{21}H_{18}ON_5Cl$	—	—	17·9	9·0	—	—	17·9	9·1
19	$C_{21}H_{18}O_3N_6$	61·8	4·5	—	—	62·7	4·5	—	—
20	$C_{23}H_{21}O_3N_5$	66·4	5·2	—	—	66·4	5·1	—	—
23	$C_{26}H_{29}O_2N_5,HCl$	64·9	6·2	14·5	—	65·0	6·3	14·6	—
24	$C_{28}H_{36}O_2N_6S,HCl$	—	—	15·2	—	—	—	15·1	—

* Cf. Table 1. [a] Found : OMe, 24·1. Reqd. : OMe, 23·8%. [b] Found : OMe, 8·1. Reqd. : 8·1%.

Preparation of Formazans.—The following illustrates the general method used : 4-Amino-3-chloroacetanilide (10 g.), dissolved in glacial acetic acid (18 c.c.), was stirred with concentrated hydrochloric acid (15 c.c.) at 0—5°. Sodium nitrite (4·5 g.) in water (5—10 c.c.) was added during 2 hr. The diazonium salt solution was added dropwise to a stirred solution of benzaldehyde phenylhydrazone (12 g.) in pyridine (90 c.c.). The temperature was kept at <12°, and the mixture was set aside for several hours. Water (250 c.c.) was added and the precipitate filtered off and washed with hot water and finally with a little methanol. It was then dried and extracted with hot benzene from which the *formazan* crystallised (Tables 1 and 3, No. 17). When the diazonium salts were soluble enough, glacial acetic acid was replaced by water and the amount of pyridine used was reduced.

Preparation of Tetrazolium Salts.—Oxidations of formazans with mercuric oxide and lead tetra-acetate were effected in methanol and chloroform respectively, either at room temperature or at the b. p., and with *iso*amyl nitrite in methanol into which a stream of hydrogen chloride was passed with stirring at 0°.

Alternative Methods of Oxidation of 1 : 3 : 5-*Triphenylformazan.*—(a) *With sodium hypochlorite.* The formazan (5 g.) was heated in glacial acetic acid (100 c.c.) under gentle reflux, and 14—18% aqueous sodium hypochlorite (*ca.* 30 c.c.) was added dropwise until the colour of the formazan disappeared. The solution was filtered and the filtrate was evaporated to dryness *in vacuo*. The crude solid was recrystallised from water, to give colourless prisms of 2 : 3 : 5-*triphenyltetrazolium chlorate*, which explodes on heating or percussion (Found: N, 14·4. $C_{19}H_{15}O_3N_4Cl$ requires N, 14·6%). A sample of the same salt prepared from 2 : 3 : 5-triphenyltetrazolium chloride and potassium chlorate had identical physical properties. Reduction of the chlorate in boiling 2N-sulphuric acid with ferrous sulphate gave, after filtration of the mixture and addition of excess of sodium bromide to the filtrate, 2 : 3 : 5-triphenyltetrazolium bromide, m. p. 239—240° (decomp.), identical with an authentic specimen.

(b) *With chromium trioxide.* The formazan (5 g.) in glacial acetic acid (50 c.c.) was treated with a solution of chromium trioxide (2 g.) in glacial acetic acid (50 c.c.) and a little water. After being boiled for 10 min., the solution was cooled and diluted with a little water, whereupon the 2 : 3 : 5-triphenyltetrazolium dichromate crystallised in orange prisms, m. p. 196—197° (decomp.). This salt also explodes on percussion. Consistent analytical data could not be obtained but the substance was identical with a specimen prepared from the chloride and potassium dichromate in acetic acid.

(c) *With hydrogen peroxide and ferrous iron.* The powdered formazan (5 g.), suspended in alcohol (50 c.c.) and 2N-sulphuric acid (20 c.c.), containing a trace of ferrous sulphate, was treated with an excess of aqueous 6% hydrogen peroxide and heated at 100° for 1 hr. Removal of alcohol by distillation, and addition of an excess of sodium bromide, precipitated crude 2 : 3 : 5-triphenyltetrazolium bromide, m. p. 240° (decomp.) after recrystallisation.

TABLE 4. *Analyses of tetrazolium salts.*

No.*		Solvent of crystn.	Found (%)				Required (%)			
			C	H	N	Hal.	C	H	N	Hal.
1	$C_{19}H_{14}O_2N_5Cl$	—	—	—	18·4	9·5	—	—	18·4	9·4
2	$C_{19}H_{14}O_2N_5Cl$	$\frac{1}{2}H_2O$	58·3	4·3	18·2	9·3	58·6	4·0	18·0	9·2
3	$C_{19}H_{14}N_4ClI$	—	49·1	3·1	12·2	27·7 ‡	49·5	3·0	12·2	27·6
4	$C_{22}H_{21}N_4Br$ [a]	C_2H_5·OH	62·0	5·5	12·6	18·4	62·2	5·4	12·6	18·0
5	$C_{19}H_{13}N_4ClBr_2$ [b]	CH_3·OH	45·8	3·2	10·7	—	45·9	3·2	10·9	—
6	$C_{20}H_{14}N_5Cl$	$\frac{1}{2}H_2O$	65·0	4·8	18·7	—	65·1	4·7	18·9	—
7	$C_{22}H_{21}O_3N_4I$ [c]	H_2O	50·3	5·0	10·7	24·0	49·5	4·3	10·5	23·7
8	$C_{25}H_{19}N_4Cl$	H_2O	70·6	5·5	13·1	8·3	70·0	4·9	13·1	8·3
9	$C_{18}H_{14}N_5Cl$ [d]	$2H_2O$	58·7	5·0	—	—	58·3	4·9	—	—
10	$C_{23}H_{18}ON_5I$ [e]	—	54·4	3·6	13·6	25·0	54·4	3·6	13·8	25·1
11	$C_{31}H_{39}N_4I$	—	—	—	9·0	20·9	—	—	9·4	21·3
12	$C_{20}H_{17}N_4Br$	—	—	—	14·2	20·2	—	—	14·2	20·4
13	$C_{32}H_{32}N_8I_2$	$4H_2O$	—	—	13·1	29·4	—	—	13·1	29·7
14	$C_{21}H_{18}ON_5Cl$	—	64·8	4·6	17·9	8·8	64·4	4·6	17·9	9·1
15	$C_{19}H_{16}N_5Cl$	$\frac{1}{2}HCl$	61·5	4·9	18·6	14·4	61·9	4·6	19·0	14·3
16	$C_{21}H_{18}ON_5Cl$	—	64·3	4·9	18·0	9·0	64·4	4·6	17·9	9·1
17	$C_{19}H_{16}N_5Cl$	C_2H_5·OH	63·8	5·6	17·5	9·4	63·7	5·6	17·7	9·0
18	$C_{27}H_{23}O_3N_6SCl$ [f]	—	—	—	15·3	6·6	—	—	15·4	6·5
19	$C_{25}H_{22}O_3N_6S$	—	61·7	4·5	17·3	—	61·7	4·7	17·1	—
20	$C_{21}H_{17}ON_5Cl_2$	H_2O	—	—	15·8	16·0	—	—	15·8	16·0
21	$C_{19}H_{15}N_5Cl_2$	$2H_2O$	—	—	16·5	17·1	—	—	16·6	16·9
22	$C_{19}H_{15}N_5Cl_2$	$\frac{1}{2}H_2O$	58·0	4·3	17·4	18·3	58·0	4·1	17·8	18·1
23	$C_{19}H_{15}O_2N_6Cl$	H_2O	55·5	4·2	20·4	8·6	55·3	4·1	20·4	8·6
24	$C_{19}H_{16}ON_5Cl$	$HCl,\frac{1}{2}H_2O$	53·4	4·9	15·5	16·0	53·1	4·7	16·3	16·5
25	$C_{31}H_{40}N_5I$	—	—	—	11·4	†	—	—	11·5	20·8
26	$C_{31}H_{41}N_6Cl$	CH_3·OH	68·4	7·8	14·8	5·6	68·1	7·9	14·8	6·3

* Cf. Table 2. † Analyses variable between 18·6 and 22·7% of I. ‡ I analysis.

[a] Found: OEt, 5·9. Reqd.: OEt, 5·1%. [b] Found: OMe, 5·9. Reqd.: OMe, 5·2%. [c] Found: OMe, 17·6. Reqd.: OMe, 17·2%. [d] Found: H_2O, 10·1. Reqd.: H_2O. 9·7%. [e] Found: OMe, 6·1. Reqd.: OMe, 6·1%. [f] Found: S, 6·5. Reqd.: S, 5·9%.

5-p-*Aminophenyl*-2 : 3-*diphenyltetrazolium Chloride.*—5-*p*-Acetamidophenyl-2 : 3-diphenyltetrazolium chloride (5 g.) was boiled under reflux for 6 hr. with aqueous hydrochloric acid (*d* 1·16). The solution was evaporated to dryness and the residue was dissolved in dilute hydrochloric acid. The free *amino-tetrazolium chloride* (Tables 2 and 4, No. 17) was precipitated by the addition of sufficient 2N-sodium hydroxide to make the solution just alkaline.

5-p-(p-*Acetamidobenzenesulphonamido*)*phenyl*-2 : 3-*diphenyltetrazolium Chloride.*—*p*-Acet-

amidobenzenesulphonyl chloride (8·5 g.) was added to a suspension of the above chloride (10 g.) in pyridine (50 c.c.). The solution was kept at room temperature for 15 min. and then warmed for a few minutes on the steam-bath. It was then cooled and diluted with water, to give a sticky orange *product* which hardened to yield a pale yellow powder (Tables 2 and 4, No. 18).

A solution of the above chloride (2 g.) in ethanol (40 c.c.) and 2N-hydrochloric acid (20 c.c.) was heated under reflux for 2 hr., then evaporated to dryness. The residue was dissolved in hot water and the solution was cooled and filtered; the solid *amino-hydroxide* was redissolved in hydrochloric acid and reprecipitated by the addition of dilute sodium hydroxide solution (Tables 2 and 4, No. 19).

3-p-*Aminophenyl*-2 : 5-*diphenyltetrazolium Chloride*.—3-*p*-Acetamidophenyl-2 : 5-diphenyltetrazolium chloride (1 g.) was heated with methanol (10 c.c.) and aqueous hydrochloric acid (*d* 1·16; 10 c.c.) overnight, to give the *amino-tetrazolium chloride* (Tables 2 and 4, No. 15).

Compounds 22—26 of Table 2 were isolated directly from the oxidation media, hydrolysis of the acetyl groups present in the formazans having been effected *in situ*, by boiling 5N-hydrochloric acid.

After the oxidation of 5-(4-*acetamido*-2-*chlorophenyl*)-1 : 3-*diphenylformazan* with mercuric oxide, the hydrolysis stage was omitted and 3-(4-*acetamido*-2-*chlorophenyl*)-2 : 5-*diphenyltetrazolium chloride* was isolated. Subsequent hydrolysis gave the *amino-tetrazolium* salt. When this oxidation procedure was followed for 5-(4-*acetamido*-3-*chlorophenyl*)-1 : 3-*diphenylformazan*, a mercuric chloride *complex* of the unacetylated 3-(4-amino-3-chlorophenyl)-2 : 5-diphenyltetrazolium chloride was isolated as pale yellow prisms, m. p. 217—219° (from methanol) [Found: C, 43·4; H, 3·1; N, 13·4; Cl, 20·0; Hg, 19·4. $(C_{19}H_{16}N_5Cl_2)_2HgCl_2$ requires C, 43·8; H, 3·1; N, 13·4; Cl, 20·4; Hg, 19·3%]. If the oxidation was followed by immediate hydrolysis with warm 5N-hydrochloric acid, the free *amino-tetrazolium* salt was obtained.

Oxidation of 5-(4-*acetamido*-2-*nitrophenyl*)-1 : 3-*diphenylformazan* with mercuric oxide gave the same type of mercuric chloride *complex*, even when acid hydrolysis followed the oxidation. The complex formed small yellow rods, m. p. 233—234·5° (decomp.), from ethanol–ether [Found: C, 42·7; H, 3·0; N, 15·5; Cl, 13·3; Hg, 18·6. $(C_{19}H_{15}O_2N_6Cl)_2HgCl_2$ requires C, 42·9; H, 2·8; N, 15·8; Cl, 13·4; Hg, 18·9%].

These complexes could be decomposed by the addition of excess of concentrated aqueous ammonia to an alcoholic solution of the complex. The solution was then filtered, and the tetrazolium salt precipitated by the addition of concentrated hydrochloric acid.

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THE RESEARCH LABORATORIES, MAY & BAKER, LTD.,
DAGENHAM, ESSEX.

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