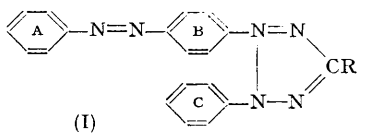
Tetrazolium Compounds. Part II. Azo-derivatives.*

By D. D. LIBMAN, A. W. NINEHAM, and R. SLACK.

[Reprint Order No. 4941.]

A new series of formazans and corresponding tetrazolium salts is described. In each compound, an *N*-phenyl group bears a phenylazo-substituent.

IN Part I * of this series, some tetrazolium salts bearing three variously substituted benzene rings were described. Of these, 3-*p*-aminophenyl-2 : 5-diphenyltetrazolium chloride showed slight activity in laboratory experiments against influenza A and Nigg mouse pneumonitis viruses,† and was chosen as a model for further study, the amino-group being replaced by phenylazo, to give (I) (R = Ph) (formal localisation of the charge on $N_{(2)}$ is not to be construed literally). The present communication deals with the preparation of substituted derivatives of (I) which could behave as biological precursors of the simpler amino-compounds. Effects of substitution in the benzene rings have been examined and two bis-tetrazolium salts have been prepared (polymethylene bridge at R).



(I)

The general synthetic methods employed are described in Part I.* Complete diazotisation of some aminoazo-compounds was difficult and was best performed in glacial acetic acid with nitrosylsulphuric acid or with sulphuric acid and a paste of sodium nitrite in water. This involved the use of larger volumes of pyridine to neutralise the excess of acid during condensations with the phenylhydrazones, but the virtual absence of water led to purer products.

Variants of ring A were introduced by the use of aminoazo-compounds prepared by (i) coupling *p*-acetamidobenzenediazonium chloride with substituted benzene derivatives, (ii) coupling diazonium salts with sodium *N*-methylaniline-ω-sulphonate, or (iii) rearrangement of diazoamino-compounds in the presence of aniline hydrochloride. Variations in ring B were obtained by application of the above methods to α-naphthylamine and *p*-xylidine. Variations in R were achieved by the use of different aldehydes and in ring C by the use of substituted phenylhydrazines.

The azoformazans did not differ markedly from the compounds described in Part I, but were a more intense purple or black and were less soluble in organic solvents. This was especially true of hydroxy-compounds. Purification was not difficult but combustion analyses for nitrogen were not always satisfactory.

Oxidations were sometimes slow but the only failures were with 1 : 3-diphenyl-5-(4-*p*-acetamidophenylazophenyl)- and 1 : 3-diphenyl-5-(4-*p*-dimethylaminophenylazophenyl)-

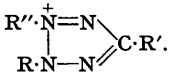
* Part I, *J.*, 1953, 3881.

† We thank Dr. R. Wien and Mr. W. F. Freeman for drawing our attention to this property of some of the tetrazolium salts described in this series of papers. Detailed biological results will be published elsewhere by our colleagues.

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

No.	R	R′	R″	Yield (%)	Appearance	M. p.	Solvent
1 [a]	Ph	Ph	*p*-C_6H_4·N:NPh	50	Purplish-black	182° *	Aq. $COMe_2$
2 [a]	Ph	Me	,,	28	Dark red	115	,,
3	Ph	Ph	*p*-C_6H_4·N:N·C_6H_4Me-*p*	53	Black, purplish-red reflex [d]	186—188	EtOAc
4	Ph	Ph	*p*-C_6H_4·N:N·C_6H_4Cl-*p*	11·5	Dark purple [d]	194·5—195	,,
5	Ph	Ph	*p*-C_6H_4·N:N·C_6H_4·NO_2-*p*	57	Purplish-black, golden reflex [e]	205—206 *	$CHCl_3$
6	Ph	Ph	*p*-C_6H_4·N:N·C_6H_4·OH-*p*	28	Dark purple, red reflex [d]	198—200	Me·NO_2
7	Ph	Ph	*p*-C_6H_4·N:N·C_6H_3Cl·OH-1 : 2 : 4	27	Dark purple, yellow reflex [f]	149—150	,,
8	Ph	Ph	*p*-C_6H_4·N:N·C_6H_3Cl·OH-1 : 3 : 4	8	Black, greenish-golden reflex	205—210	,,
9	Ph	Ph	4-Phenylazo-1-naphthyl	9 [b]	Indigo-blue [d]	200	$CHCl_3$
10	Ph	*p*-C_6H_4·OH	*p*-C_6H_4·N:NPh	50	Dark purple [d]	180	MeOH
11	Ph	Ph	2 : 5 : 4-$C_6H_2Me_2$·N:NPh	50	Dark purple, green reflex [d]	197 *	EtOAc
12	*p*-C_6H_4·CO_2H	Ph	*p*-C_6H_4·N:NPh	10	Black, green reflex [f]	209	,,
13 [a]	*p*-C_6H_4Cl	Ph	,,	18	Purplish-black	168—170	Aq. $COMe_2$
14 [a]	*p*-C_6H_4·NHAc	Ph	,,	26	Purple	215	,,
15 [c]	Ph	$-[CH_2]_2-$	,,	29	Purple	136—138	*cyclo*Hexane
16 [c]	Ph	$-[CH_2]_6-$	,,	39	Purplish-black [e]	161—162 *	C_6H_6–*cyclo*hexane
17	Ph	Ph	*p*-C_6H_4·N:N·C_6H_4·NHAc-*p*	35	Dark red [e]	216	$CHCl_3$–MeOH
18	Ph	Ph	*p*-C_6H_4·N:N·C_6H_4·NMe_2-*p*	23	Dark purple	182	Me·NO_2
19	*p*-C_6H_4·NHAc	*p*-C_6H_4·NHAc	*p*-C_6H_4·N:N·C_6H_4·OH-*p*	small	Dark purple	188	MeOH
20	Ph	Ph	2 : 5-Dimethyl-4-2′-thiazolylazo-phenyl	25	Violet [d]	216 *	$CHCl_3$
21	Ph	*p*-C_6H_4·OAc	*p*-C_6H_4·N:NPh	50	Dark purple [d]	191	EtOAc

[a] Prepared by (Miss) B. M. Davies. [b] Overall yield from aniline and α-naphthylamine. [c] This is the bis-compound. [d] Needles. [e] Prisms. [f] Plates. * With decomp.

TABLE 2. *Tetrazolium salts*,  .

No.*	Yield (%)	Anion	Method of oxidn.	Appearance	M. p. †	Solvent
1	24	I	B	Orange	231—232°	MeOH
2	—	I	B	Orange needles	127—129	EtOH–Et_2O
3	36	I	A	Ruby-red prisms	175—177	EtOH
4	72	Cl	A	Red needles	184—185	EtOH–Et_2O
5	45	Cl	B	Orange-red prisms	250	Aq. EtOH
6	70	Cl	C	Orange-red prisms	230	EtOH–Et_2O
7	26	Cl	C	Dark red prisms	204—205	,,
8	53	Cl	C	Dark orange-red prisms	206—207	,,
9	48	I	B	Orange needles	280	Aq. EtOH
10	97	Cl	C	Orange-brown rhombs	267	EtOH
11	54	Cl	A	Orange prisms	41	H_2O
12	48	Cl	A	Red prisms	264	MeOH
13	33	I	B	Orange	219	MeOH–Et_2O
14	40	I	B	Orange	258	EtOH–Et_2O
15	69	I	B	Dark red	185—186	EtOH
16	24	I	A	Red prisms	169—170	,,

Methods of oxidation: A, Mercuric oxide in methanol; B, lead tetra-acetate in chloroform; C, *iso*amyl nitrite and hydrogen chloride.

* The tetrazolium salts have the R, R′, and R″ of the formazans of corresponding number in Table 1. † With decomp., except no. 13.

formazan. In these cases undesirable secondary oxidations can occur. Most of the tetrazolium salts were sparingly soluble in water. The isethionates, into which some iodides were converted, were not markedly more soluble than the halides. The new salts and the corresponding formazans are listed in Tables 1 and 2.

Experimental

p-*Acetoxybenzaldehyde phenylhydrazone* formed triangular plates (from ethanol) (95%), m. p. 154° (Found: N, 10·7. $C_{15}H_{14}O_2N_2$ requires N, 11·0%).

p-*Acetamidobenzaldehyde* p-*Acetamidophenylhydrazone*.—*p*-Acetamidophenylhydrazine stannichloride (Franzen and von Fürst, *Annalen*, 1916, **412**, 41) (100 g., 0·2 mol.) and crystalline sodium acetate (100 g.) in hot water (1 l.) were heated with a solution of *p*-acetamidobenzaldehyde (33 g., 0·2 mol.) in aqueous methanol at 95° for 30 min., to give the *phenylhydrazone* as cream-coloured needles (40%), m. p. 233° (from aqueous methanol) (Found: N, 18·3. $C_{17}H_{18}O_2N_4$ requires N, 18·1%).

Table 3. *Analyses of formazans.*

		Found (%)				Required (%)			
No.*	Formula	C	H	N	Cl	C	H	N	Cl
1	$C_{25}H_{20}N_6$	74·2	5·3	20·0	—	74·3	5·0	20·8	—
2	$C_{20}H_{18}N_6,H_2O$	66·9	5·6	23·1	—	66·7	5·6	23·3	—
3	$C_{26}H_{22}N_6$	74·3	5·0	—	—	74·6	5·3	—	—
4	$C_{25}H_{19}N_6Cl$	—	—	18·9	7·8	—	—	19·1	8·1
5	$C_{25}H_{19}O_2N_7$	67·0	4·2	21·5	—	66·8	4·2	21·8	—
6	$C_{25}H_{20}ON_6$	70·4	5·0	19·4	—	71·4	4·8	20·0	—
7	$C_{25}H_{19}ON_6Cl$	—	—	17·9	7·0	—	—	18·5	7·8
8	$C_{25}H_{19}ON_6Cl$	—	—	—	7·1	—	—	—	7·8
9	$C_{29}H_{22}N_6$	—	—	18·4	—	—	—	18·5	—
10	$C_{25}H_{20}ON_6,\frac{1}{2}CH_3{\cdot}OH$	70·6	5·0	18·6	—	70·2	5·0	19·3	—
11	$C_{27}H_{24}N_6$	75·2	5·6	19·1	—	75·0	5·6	19·4	—
12	$C_{26}H_{20}O_2N_6$	69·7	5·2	18·2	—	69·6	4·5	18·7	—
13	$C_{25}H_{19}N_6Cl$	68·7	4·5	—	8·0	68·5	4·3	—	8·1
14	$C_{27}H_{23}ON_7$	70·2	4·9	—	—	70·2	5·0	—	—
15	$C_{40}H_{34}N_{12}$	70·5	5·2	23·1	—	70·5	5·0	24·6	—
16	$C_{44}H_{42}N_{12},C_6H_{12}$	72·1	6·3	20·5	—	71·9	6·5	20·4	—
17	$C_{27}H_{23}ON_7$	69·7	5·0	20·9	—	70·2	5·0	21·2	—
18	$C_{27}H_{25}N_7$	72·2	5·7	21·2	—	72·5	5·6	21·9	—
19	$C_{29}H_{26}O_3N_6,2CH_3{\cdot}OH$	62·0	5·2	—	—	62·0	5·0	—	—
20	$C_{24}H_{21}N_7S$	—	—	21·6	6·8 †	—	—	22·3	7·3 †
21	$C_{27}H_{22}O_2N_6$	70·5	5·0	17·8	—	70·1	4·8	18·2	—

* Cf. Table 1. † Sulphur analysis.

Table 4. *Analyses of tetrazolium salts.*

		Found (%)				Required (%)			
No.*	Formula	C	H	N	Hal.	C	H	N	Hal.
1	$C_{25}H_{19}N_6I$	56·4	3·6	—	23·9	56·5	3·6	—	24·0
2	$C_{20}H_{17}N_6I$	51·2	4·1	—	27·0	51·3	3·6	—	27·1
3	$C_{26}H_{21}N_6I$	56·0	3·8	15·7	23·9	57·4	3·8	15·4	23·3
4	$C_{25}H_{18}N_6Cl_2,1{\cdot}5H_2O$	60·3	4·4	16·8	14·2	60·0	4·2	16·8	14·2
5	$C_{25}H_{18}O_2N_7Cl,2H_2O$	57·8	4·5	19·0	6·7	57·8	4·2	18·8	6·8
6	$C_{25}H_{19}ON_6Cl,\frac{1}{2}H_2O$	64·6	4·3	17·9	7·5	64·7	4·3	18·1	7·6
7	$C_{25}H_{18}ON_6Cl_2,H_2O$	59·5	4·1	16·2	13·7	59·2	3·6	16·6	14·0
8	$C_{25}H_{18}ON_6Cl_2,C_2H_5{\cdot}OH$	60·2	4·7	15·8	13·0	60·5	4·5	15·7	13·2
9	$C_{29}H_{21}N_6I$	—	—	14·2	22·0	—	—	14·5	21·9
10	$C_{25}H_{19}ON_6Cl$	—	—	19·0	7·4	—	—	18·5	7·8
11	$C_{27}H_{23}N_6Cl,3H_2O$	—	—	16·3	6·8	—	—	16·2	6·8
12	$C_{26}H_{19}O_2N_6Cl,\frac{1}{2}H_2O$	63·2	4·2	16·6	6·8	63·4	4·1	17·0	7·0
13	$C_{25}H_{18}N_6ClI$	52·8	3·4	—	22·4 †	53·1	3·2	—	22·5 †
14	$C_{27}H_{22}ON_7I$	55·0	3·8	—	21·6	55·1	3·8	—	21·6
15	$C_{40}H_{32}N_{12}I_2,C_2H_5{\cdot}OH$	—	—	16·5	25·8	—	—	17·1	25·9
16	$C_{44}H_{40}N_{12}I_2,H_2O$	—	—	16·7	25·3	—	—	16·7	25·3

* Cf. Table 2. † Iodine analysis.

4-p-*Aminophenylazo*-2-*chlorophenol*.—Diazotised *p*-acetamidoaniline was coupled with *o*-chlorophenol. Hydrolysis with hydrochloric acid gave the *aminoazo*-compound (51%), m. p.

186—187° (from aqueous ethanol) (Found: C, 58·7; H, 4·3; N, 17·2. $C_{12}H_{10}O_3NCl$ requires C, 58·2; H, 4·3; N, 16·9%).

4-p-*Aminophenylazo-3-chlorophenol*, prepared in the same way, formed orange plates (83%), m. p. 165°, from aqueous ethanol (Found: N, 15·8; Cl, 13·6. $C_{12}H_{10}ON_3Cl,H_2O$ requires N, 15·8; Cl, 13·4%). The N-*acetyl* derivative, golden needles (from ethanol), had m. p. 213—215° (Found: N, 14·2. $C_{14}H_{12}O_2N_3Cl$ requires N, 14·5%).

2-p-*Aminophenylazothiazole*.—Diazotised 2-aminothiazole was coupled with sodium *N*-methylaniline-ω-sulphonate in alkaline solution (cf. Elbs, *J. pr. Chem.*, 1924, [2], **108**, 229). Hydrolysis of the intermediate sulphonate with hot 50% sodium hydroxide solution gave the *aminoazo*-compound (36%), dark red rhombs or stout prisms, m. p. 188—189° (from toluene) (Found: N, 27·3. $C_9H_8N_4S$ requires N, 27·5%). The N-*acetyl* compound, m. p. 231°, formed orange needles (88%) from 50% ethanol (Found: N, 22·0. $C_{11}H_{10}ON_4S$ requires N, 22·8%).

2 : 5-*Dimethyl-4-2′-thiazolylazoaniline* was obtained as dark red prisms with a green reflex (from benzene) or as crimson needles [from benzene–light petroleum (b. p. 60—80°) (1 : 1)], m. p. 158° (28%) (Found: C, 57·8; H, 5·2; N, 23·0; S, 14·0. $C_{11}H_{12}N_4S$ requires C, 57·0; H, 5·2; N, 24·1; S, 13·8%). The N-*acetyl* derivative formed orange prisms or yellow needles, m. p. 187° (from benzene) (Found: N, 20·2; S, 12·1. $C_{13}H_{14}ON_4S$ requires N, 20·3; S, 11·6%).

4-2′-*Thiazolylazo-1-naphthylamine* formed purple needles (from 60% ethanol), m. p. 195° (60%) (Found: N, 21·5. $C_{13}H_{10}N_4S$ requires N, 22·0%). The N-*acetyl* derivative formed brown needles (60%), m. p. 236° (from ethanol) (Found: N, 18·8. $C_{15}H_{12}ON_4S$ requires N, 18·9%).

The following formazans were not isolated from the appropriate condensations: 1 : 3-diphenyl-5-*p*-(*p*-sulphophenylazo)phenyl-, 5-(4-*p*-hydroxyphenylazo-1-naphthyl)-1 : 3-diphenyl-, 3-*p*-acetoxyphenyl-5-*p*-(4-hydroxyphenylazo)phenyl-1-phenyl-, 1 : 3-diphenyl-5-(4-2′-thiazolylazophenyl)-, 5-[2 : 5-dichloro-4-(2 : 5-dichlorophenylazo)phenyl]-1 : 3-diphenyl-, 1 : 3-diphenyl-5-(1-phenylazo-2-naphthyl)-, 1 : 3-diphenyl-5-(4-1′-naphthylazo-1-naphthyl)-, 1 : 3-diphenyl-5-(4-2′-thiazolylazo-1-naphthyl)-, and 1-*p*-nitrophenyl-3-phenyl-5-*p*-phenylazophenyl-formazan, octamethylenebis-3-(1-phenyl-5-*p*-phenylazophenylformazan) and octamethylenebis-3-(1-*p*-nitrophenyl-5-*p*-phenylazophenylformazan).

We thank Dr. A. J. Ewins, F.R.S., for his interest, Mr. S. Bance, B.Sc., and his staff for semi-microanalyses, and the Directors of May & Baker Ltd. for permission to publish these results.

Research Laboratories, May & Baker Ltd.,
Dagenham, Essex.

[*Received, December 30th*, 1953.]