276. *Aryl-2-halogenoalkylamines. Part XVI.* The Preparation of Derivatives of 4-[Di-(2-chloroalkyl)amino]azobenzenes.*

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The preparation is described of certain mono-, di-, and tri-substituted derivatives of 4-[di-(2-chloroalkyl)amino]azobenzenes. A brief report on the activity of the compounds as tumour-growth inhibitors is given.

In Part XIV [1] was outlined a new approach to the preparation of potentially cytotoxic compounds. This concerned aryldi-(2-chloroethyl)amines in which the chlorine atoms were relatively unreactive but which would become activated in a chemical sense if the molecule underwent a change such as could readily occur *in vivo*. Derivatives of the azo-compound (I) appeared to be particularly suitable for investigation since most of the parent substances contain chlorine atoms of low chemical reactivity whereas reduction products (hydrazo-compounds or amines), possibly formed in the organism,[2] would certainly be more reactive. This compound and its 2′-carboxy-derivative are known to inhibit the growth of the transplanted Walker rat carcinoma, whereas the 4′-nitro- and 3′- and 4′-carboxy-derivatives are inactive.[3] Accordingly a large number of substituted 4-[di-(2-chloroalkyl)amino]azobenzenes has now been synthesised and examined.

* Part XV, *J.*, 1955, 3835.

[1] Ross, Warwick, and Roberts, *J.*, 1955, 3110.
[2] Hamon, *Ann. Chim.* (*France*), 1947, **2**, 233.
[3] Haddow, *Ann. Report Brit. Empire Cancer Campaign*, 1952, **30**, 28; 1953, **31**, 9; Everett, Roberts, and Ross, *J.*, 1953, 2386.

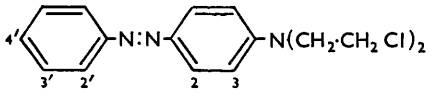
Most of the compounds were prepared by coupling the diazonium salt obtained from the appropriate amine with *NN*-di-(2-chloroethyl)aniline or with its *o*- or *m*-substituted derivative. It was not possible to prepare a stable diazonium salt from *NN*-di-(2-chloroethyl)-*p*-phenylenediamine and so the alternative route employing this intermediate could not be followed.

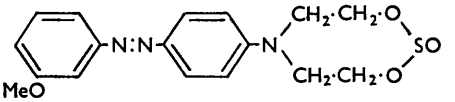
Our results support the generally accepted view that the diazonium cation is an electrophilic reagent and that electron-releasing substituents *ortho* to the point of coupling facilitate reaction. For example, all the 2-methyl and 2-methoxy-derivatives of compound (I) were very readily obtained—even the diazonium salt derived from *o*-anisidine which did not couple with *NN*-di-(2-chloroethyl)aniline gave these 2-substituted compounds. On the other hand, an *ortho*-carboxy-group hindered the reaction and only the powerfully coupling salts derived from *p*-nitroaniline and *o*-cyanoaniline yielded 2-carboxy-derivatives of (I). The 2 : 2′-dicarboxy-derivative could not be obtained by direct coupling or by acid hydrolysis of the 2-carboxy-2′-cyano-derivative; in this case as elsewhere vigorous acid treatment resulted in the decomposition of the azo-compound.[4]

NN-Di-(2-chloroethyl)-*o*-anisidine did not couple very readily but it afforded 4′-carboxy-4-[di-(2-chloroethyl)amino]-3-methoxyazobenzene in low yield. The low reactivity of *ortho*-substituted arylamines in coupling reactions [5] is considered to be due to the steric restriction placed on the coplanarity of the substituted amino-group with the benzene ring with consequent reduction of electron density at the *para*-coupling position.

It is well established that electron-attracting groups in the aromatic ring of the diazonium compound aid coupling whilst electron-releasing groups hinder it. In accordance, it is now found that whereas the diazonium salt from *m*-toluidine and *m*-anisidine coupled readily with *NN*-di-(2-chloroethyl)aniline the *ortho*- and *para*-isomers required more vigorous conditions : with *o*-anisidine no coupling has occurred under the conditions so far used. Some difficulty was experienced in preparing the 2′-phenyl derivative of (I), but this is probably due to steric hindrance of the coupling reaction. Diazonium salts from the chloro-, bromo-, and nitro-anilines and from *p*-acetylaniline coupled very readily.

Although the diazonium salt from *p*-anisidine did not couple very easily with *NN*-di-(2-chloroethyl)aniline it did so with the more basic *NN*-di-(2-hydroxyethyl)aniline. It was hoped to obtain the required chloroethylamine in better yield by the action of phosphoryl chloride or phosphorus pentachloride on the hydroxyethylaminoazobenzene but experiments with 4-[di-(2-hydroxyethyl)amino]-3′-methoxyazobenzene showed that charring and loss of azo-character occurred. It was considered that this might be due to the acid reaction conditions and so an attempt was made to prepare the chloro-derivative by the action of thionyl chloride in pyridine : the product was apparently the cyclic sulphite (II).

(I) 

(II) 

Diazotisation of 2-hydroxy-5-nitroaniline gave a red diazo-oxide which was soluble in methanolic hydrochloric acid. There was little indication of coupling when *NN*-di-(2-chloroethyl)aniline was added to this solution at 0° but 2 hours' heating at 40—50° gave a 25% yield of the required 2′-hydroxy-5′-nitro-derivative. The diazonium compound appeared to be quite stable at this elevated temperature for no appreciable evolution of nitrogen was observed.

Hydrolysis Rates.—The rates of hydrolysis of a selection of the new compounds under the standard conditions [6] have been measured. All but one of the compounds examined have very low chemical reactivity (see Table). The exception is the 4-[di-(2-chloroethyl)-amino]-3-methoxy-derivative whose high reactivity is a consequence of the greater basicity

[4] Cf. Jacobson, *Annalen*, 1909, **367**, 304.
[5] Cf. Friedländer, *Monatsh.*, 1898, **19**, 627; Bamberger, *Ber.*, 1895, **28**, 243; Bamberger and Meimberg, *ibid.*, p. 1891; Gnehm and Blumer, *Annalen*, 1899, **304**, 87.
[6] Ross, *J.*, 1949, 183.

Substituted 4-[di-(2-chloroalkyl)amino]azobenzenes of type (I).

Substituent	Method	M. p.	Solvent	Form	Formula	Found (%): C	H	N	Required (%): C	H	N	Hydrolysis rate[i]	Activity[h]
None[a]	A(i)	73—75°	A	Orange plates	$C_{16}H_{17}N_3Cl_2$	—	—	—	—	—	—	<1%	+ve
2-Me	A(i)	87·5—89	C	Orange prism. needles	$C_{17}H_{19}N_3Cl_2$	60·2	5·7	12·5	60·7	5·7	12·5		(low)
2′-Me	B(ii)[b]	97	A	Red needles	,,	60·6	5·9	12·5	60·7	5·7	12·5		+ve
3′-Me	B(i)	76	A	Orange needles	,,	60·7	5·9	12·6	60·7	5·7	12·5		−ve
4′-Me	B(i)	84	A	Orange needles	,,	60·4	5·8	12·6	60·7	5·7	12·5		−ve
2-MeO	A(i)	79—80	B	Orange plates	$C_{17}H_{19}ON_3Cl_2$	58·2	5·6	11·9	57·9	5·4	11·9		+ve
3′-MeO	B(i)	64—66	J—B	Orange needles	,,	58·0	5·6	12·4	57·9	5·4	11·9		−ve
4′-MeO	B(ii)	104—105	H	Orange needles	,,	57·7	5·5	12·2	57·9	5·4	11·9		−ve
2′-Cl	A(i)	109	H	Red needles	$C_{16}H_{16}N_3Cl_3$	53·5	4·8	12·0	53·9	4·5	11·8		−ve
3′-Cl	A(i)	84	H	Orange plates	$C_{16}H_{16}N_3Cl_3$	53·5	4·7	11·8	53·9	4·5	11·8		−ve
4′-Cl	A(i)	130	H	Golden plates	$C_{16}H_{16}N_3Cl_3$	54·0	4·7	11·7	53·9	4·5	11·8		−ve
2′-Br	A(i)	97—100	H	Orange needles	$C_{16}H_{16}N_3Cl_2Br$	48·0	4·3	10·4	47·9	4·0	10·5		−ve
3′-Br	A(i)	76—78	E—H	Red-orange needles	$C_{16}H_{16}N_3Cl_2Br$	47·9	4·0	10·3	47·9	4·0	10·5		−ve
4′-Br	A(i)	132—134	E—B	Golden needles	$C_{16}H_{16}N_3Cl_2Br$	47·6	4·3	10·7	47·9	4·0	10·5		−ve
2′-I	A(i)	97—98	H	Brown needles	$C_{16}H_{16}N_3Cl_2I$	42·7	3·8	9·4	42·8	3·6	9·4		−ve
2′-CN	A(i)	114—115	H	Red needles	$C_{17}H_{16}N_4Cl_2$	59·3	4·8	15·9	58·8	4·6	16·1		(low)
2′-NO_2	A(i)	119	H	Red needles	$C_{16}H_{16}O_2N_4Cl_2$	52·4	4·5	15·2	52·3	4·4	15·3		−ve
3′-NO_2	A(i)	113	H	Orange needles	$C_{16}H_{16}O_2N_4Cl_2$	52·3	4·6	15·6	52·3	4·4	15·3		−ve
4′-NO_2[a]	A(i)	166—167	F	Red plates	$C_{16}H_{16}O_2N_4Cl_2$	—	—	—	—	—	—	<1%	−ve
2′-CO_2H[d]	A(i)	179—180	F	Orange-red plates	$C_{17}H_{17}O_2N_3Cl_2$	—	—	—	—	—	—	<1%	+ve
3′-CO_2H[d]	A(i)	162—164	G	Yellow needles	,,	—	—	—	—	—	—	<1%	−ve
4′-CO_2H[d]	A(i)	212—214	K	Orange-red plates	,,	—	—	—	—	—	—	<1%	−ve
4′-Ac	A(ii)	136	H	Golden plates	$C_{18}H_{19}ON_3Cl_2$	59·5	5·5	12·1	59·3	5·3	11·5		−ve
2′-Ph	A(i)	92	H—B	Red prisms	$C_{22}H_{21}N_3Cl_2$	65·8	5·5	10·6	66·3	5·3	10·6		−ve
4′-Ph	A(i)	142	B	Golden needles	$C_{22}H_{21}N_3Cl_2$	65·9	5·6	10·6	,,	,,	,,		−ve
2′-SO_3H	C	Indef.	g	Blue needles	$C_{16}H_{17}O_3N_3SCl_2$	47·6	4·5	10·3	47·9	4·3	10·5	<1%	−ve
4′-SO_3H	C	Indef.	g	Blue needles	,,	47·6	4·4	10·3	,,	,,	,,	<1%	−ve
2′-CO_2Me[e]	A(ii)	107—108	H	Red needles	$C_{18}H_{19}O_2N_3Cl_2$	56·6	5·2	11·1	56·9	5·1	11·1		+ve
2′ : 3′-Benzo	B(ii)[f]	103	A	Red prisms	$C_{20}H_{19}N_3Cl_2$	64·8	5·3	11·1	64·5	5·1	11·3		−ve
Analogues containing $N(CH_2{\cdot}CHMeCl)_2$.													
2′-NO_2	A(i)	104	H	Red needles	$C_{18}H_{20}O_2N_4Cl_2$	55·0	5·2	14·3	54·7	5·1	14·2		−ve
4′-NO_2	A(i)	169	E—B	Red needles	$C_{18}H_{20}O_2N_4Cl_2$	54·7	5·3	13·8	54·7	5·1	14·2		−ve

Table continues on opposite page.

Substituted 4-[*di*-(2-*chloroalkyl*)*amino*]*azobenzenes of type* (I). (*Continued.*)

Substituent	Method	M. p.	Solvent	Form	Formula	Found (%): C	H	N	Required (%): C	H	N	Hydrolysis rate[i]	Activity[h]
Analogue containing $NEt\cdot CH_2\cdot CH_2Cl$.													
2′-CO_2H	A(i)	145·5—147·5°	G	Red needles	$C_{17}H_{18}O_2N_3Cl$	61·1	5·3	12·8	61·5	5·5	12·6		−ve
2-MeO : 2′-Me	A(i)	99—101	B	Red needles	$C_{18}H_{21}ON_3Cl_2$	59·3	6·1	11·2	59·0	5·8	11·5		+ve
2-Me : 4′-MeO	A(ii)	98	H	Orange plates	,,	58·8	5·8	11·6	59·0	5·8	11·5	<1%	+ve
2-MeO : 2′-MeO	A(i)	138—139	E—A	Red-orange needles	$C_{18}H_{21}O_2N_3Cl_2$	56·4	5·7	11·2	56·6	5·5	11·0		(low)
2-MeO : 2′-OH	A(ii)	136—138	H	Brown needles	$C_{17}H_{19}O_2N_3Cl_2$	55·4	5·3	11·5	55·4	5·2	11·4		−ve
2-Me : 2′-Cl	A(i)	122—123	H	Orange needles	$C_{17}H_{18}N_3Cl_3$	54·8	5·0	11·6	55·1	4·9	11·3		−ve
2′-Me : 3′-Cl	A(i)	98—99	H	Red-brown needles	$C_{17}H_{18}N_3Cl_3$	55·2	5·2	11·4	55·1	4·9	11·3		−ve
2′-Me : 4′-Cl	A(i)	78—80	H	Tan needles	,,	54·6	5·1	11·5	55·1	4·9	11·3		−ve
2′ : 3′-Cl_2	A(i)[f]	135	H	Red needles	$C_{16}H_{15}N_3Cl_4$	48·9	4·1	10·8	49·1	3·9	10·8	<1%	−ve
2′ : 4′-Cl_2	A(i)[f]	109—110	H	Orange-red needles	,,	49·1	4·2	10·8	49·1	3·9	10·8		−ve
3′ : 4′-Cl_2	A(i)[f]	88	H	Golden plates	,,	49·3	4·1	10·6	49·1	3·9	10·8		−ve
2-Me : 2′-NO_2	A(i)	148	H	Red needles	$C_{17}H_{18}O_2N_4Cl_2$	53·5	4·8	14·6	53·6	4·8	14·7		−ve
3-MeO : 3′-NO_2	A(i)	120—121	H	Red needles	$C_{17}H_{18}O_3N_4Cl_2$	51·6	4·8	14·1	51·3	4·6	14·1		
3-MeO : 4′-NO_2	A(i)	131—132	H	Red needles	$C_{17}H_{18}O_3N_4Cl_2$	51·3	4·9	14·3	51·3	4·6	14·1		
2′ : 4′-$(NO_2)_2$	A(i)[j]	186—189	H	Red needles	$C_{16}H_{15}O_4N_5Cl_2$	47·0	3·8	17·1	46·6	3·7	17·0		−ve
2′-OH : 5′-NO_2	D	166	E	Red needles	$C_{16}H_{16}O_3N_4Cl_2$	50·2	4·3	14·3	50·1	4·2	14·6		(low)
2-Me : 2′-CO_2H	A(i)	190—191	F	Red needles	$C_{18}H_{19}O_2N_3Cl_2$	56·6	5·1	10·8	56·8	5·0	11·1	<1%	+ve
2-Me : 4′-CO_2H	A(i)	223	H	Orange needles	$C_{18}H_{19}O_2N_3Cl_2$	56·6	5·1	11·4	56·8	5·0	11·1	<1%	+ve
2-MeO : 2′-CO_2H	A(i)	182	H, F	Orange needles	$C_{18}H_{19}O_3N_3Cl_2$	54·8	5·0	10·8	54·6	4·8	10·6	<1%	+ve
3-MeO : 3′-CO_2H	A(i)	132—134	B, D	Orange prisms	$C_{18}H_{19}O_3N_3Cl_2$	54·9	5·0	10·3	54·6	4·8	10·6		
3-MeO : 4′-CO_2H	—	166	G	Orange prism. needles	$C_{18}H_{19}O_3N_3Cl_2$	54·6	4·9	10·7	54·6	4·8	10·6	Acid 35% Na salt 21%	+ve
2-CO_2H : 4′-NO_2	A(i)	185—190[k]	E	Purple plates	$C_{17}H_{16}O_4N_4Cl_2$	50·0	4·1	13·1	49·7	3·9	13·6	<1%	+ve
2-CO_2Me : 4′-NO_2	A(i)	135—138	D	Orange plates	$C_{18}H_{18}O_4N_4Cl_2$	50·4	4·3	13·2	51·4	4·6	13·2		(low)
2-CO_2H : 4′-CO_2H	A(ii)	197	H	Orange powder	$C_{18}H_{17}O_4N_3Cl_2$	52·9	4·0	9·9	52·7	4·2	10·2		+ve
2-CO_2H : 2′-CN	A(i)	213—214	J	Red needles	$C_{18}H_{16}O_2N_4Cl_2$	55·3	4·4	14·4	55·3	4·1	14·3	<1%	+ve
2-Me : 2′ : 3′-benzo	—	118—119	G	Red-purple needles	$C_{21}H_{21}N_3Cl_2$	65·2	5·7	10·8	65·2	5·7	10·9		−ve
2-Me : 4′-Me : 2′-CO_2H	A(i)	204	F	Orange needles	$C_{19}H_{21}O_2N_3Cl_2$	57·7	5·5	10·4	57·9	5·4	10·7	<1%	+ve
2-Me : 2′-OH : 5′-NO_2	D	193—194	E	Red needles	$C_{17}H_{18}O_3N_4Cl_2$	51·5	4·7	14·2	51·4	4·6	14·1		−ve

Solvents used for crystallisation are: A, light petroleum (b. p. 40—60°); B, light petroleum (b. p. 60—80°); C, pentane; D, *cyclo*hexane; E, benzene; F, acetone; G, methanol; H, ethanol; J, ethyl acetate; K, 2-methoxyethanol.

[a] Everett and Ross, *J.*, 1949, 1972. [b] Reaction time 2 weeks. [c] Reaction time 5 weeks. [d] Ref. 3. [e] Also prepared by the action of diazomethane on the acid. [f] Reaction time 1 week. [g] See Experimental section. [h] As inhibitors of the growth of the transplanted Walker rat carcinoma. [i] Diazotisation of amine as outlined by Saunders (ref. 7) see also Noelting and Kopp, *Ber.*, 1905, **38**, 3506. [j] Diazotisation of amine as outlined by Saunders (ref. 7). [k] Dependent on the rate of heating. [l] Based on the development of acidity.

of the amino-group due to hindrance of coplanarity with the benzene ring. The stronger basic character is also reflected in the absorption spectrum which will be discussed in a subsequent paper.

Biological Activity.—Monosubstitution in the 3′- and 4′-position of the active parent substance (I) leads in all the examples studied to loss of the biological activity (Table). However, the incorporation of electron-releasing groups into the molecule at the positions *ortho* to the azo-linkage does not lead to deactivation. The 2′-carboxy-derivative comes into this category for its acidic group will be ionised under physiological conditions; the corresponding methyl ester almost certainly owes its activity to hydrolysis *in vivo*. Activity in compounds bearing electron-releasing *ortho*-substituents is confirmed by the results shown in the Table. One of the most effective compounds is the 2′-carboxy-2-methyl-derivative which contains two such substituents. It will be shown in a later paper that *ortho*-substitution often facilitates the reduction of the azo-linkage in neutral solutions. With the exception of the unsubstituted compound (I) and its 4′-carboxy-3-methoxy-derivative all the biologically active compounds have at least one substituent in a position *ortho* to the azo-linkage. Provided that such a substituent is present further substitution in the 3′- and 4′-position does not normally lead to loss of activity. On account of its higher chemical reactivity (see above) 4′-carboxy-4-[di-(2-chloroethyl)amino]-3-methoxy-azobenzene differs from all the other azo-derivatives now described in that reductive fission of the azo-linkage is probably not necessary to produce an active compound.

Experimental

Preparation of Azo-compounds.—Method A (i). The amine (0·02 mole), in concentrated hydrochloric acid (6 ml.) and water (10 ml.), was converted into the diazonium salt by addition of sodium nitrite (0·02 mole) in water (5 ml.), then added with stirring to a solution of the appropriate aryldi(chloroalkyl)amine (0·02 mole) in ethanol (150 ml.) at 10°. After 12 hr. at 0° the product was collected.

Method A (ii). As A (i), but the reaction time was one week.

Method B (i). The amine (0·08 mole) in concentrated hydrochloric acid (24 ml.) and water (25 ml.) was converted into the diazonium salt by the addition of sodium nitrite (0·08 mole) in water (15 ml.), and ethanol (100 ml.) was added. The aryldi(chloroalkyl)amine (0·02 mole) in ethanol (100 ml.) was added dropwise during 1 hr. to the ice-cooled solution. Stirring was continued for 3 hr. and then the mixture was left overnight at 0°.

Method B (ii). As B (i) but the reaction time was 1—5 weeks.

Method C (for the preparation of the azo-sulphonic acids). The precipitated diazonium salt obtained from the amino-sulphonic acid (0·02 mol.) as described by Saunders [7] was suspended in ethanol (100 ml.) and to this was added the aryldi(chloroalkyl)amine (0·02 mol.) in ethanol (150 ml.). Stirring was continued at room temperature for 3 days and then the azo-compound was collected. The azo-sulphonic acids were crystallised from the minimum amount of hot water rendered alkaline by sodium hydroxide (2N). Addition of an excess of hydrochloric acid (10N) to the hot filtered solution, followed by slow cooling, gave deep blue needles of indefinite m. p.

Method D. The nitro-amine was diazotised as in method A (i). The precipitated diazo-oxide was dissolved in concentrated hydrochloric acid (20 ml.), and methanol (50 ml.) was added. The filtered solution was poured into a solution of the aryldi(chloroalkyl)amine (0·02 mole) in methanol (125 ml.), heated at 40—50° for several hours, then kept at 0° overnight; the azo-compound separated.

The *products* are recorded in the Table.

Attempted Conversion of 4-Amino-NN-di-(2-hydroxyethyl)-3′-methoxyazobenzene into the Di-2″-chloroethyl Derivative.—Thionyl chloride (2 ml.) was added to a cooled solution of the hydroxyethylaminoazo-compound (2 g.) in pyridine (20 ml.). After ¾ hr. at room temperature the mixture was heated for 5 min. at 90°, then evaporated to dryness under reduced pressure. A benzene solution of the residue was passed through a column of activated alumina. The eluates contained an orange solid which after several crystallisations from ethanol gave orange needles, m. p. 149·5° (Found : C, 56·0; H, 5·3; N, 11·5; S, 9·3. $C_{17}H_{19}O_4N_3S$ requires C, 56·5; H, 5·3; N, 11·6; S, 8·9%), which appeared to be the *cyclic sulphite* (II).

[7] Saunders, " The Aromatic Diazo-compounds," Arnold, London, 1949, p. 10.

Hydrolysis Rates of the Di(chloroethyl)aminoazo-derivatives.—These were determined as described by Ross,[6] the least volume of 50% aqueous acetone being used to effect solution. The acidity developed during the hydrolysis was determined by potentiometric titration.

This investigation was supported by grants to this Institute from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service, and was carried out during the tenure of one of us (G. P. W.) of an overseas C.S.I.R.O. research studentship. The authors thank Professor A. Haddow for permission to quote the results of tumour inhibition studies.

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