105. *Synthesis of Lipophilic Chemotherapeuticals. Part II. 4-Alkylaminoazobenzene-4'-arsonic Acids.*

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In continuation of the programme outlined in Part I (J., 1939, 1) we have studied the effect on the biological properties of 4-aminoazobenzene-4'-arsonic acid of introducing alkyl groups of increasing molecular weight into the amino-group.

A SERIES of dyes, $R{\cdot}NH{\cdot}C_6H_4{\cdot}N{:}N{\cdot}C_6H_4{\cdot}AsO_3H_2$, was prepared by coupling diazotised *p*-arsanilic acid with a solution of the substituted aniline, usually in glacial acetic acid. The lower members of the series are very toxic, the higher ones where R = $C_{12}H_{25}$, $C_{14}H_{29}$, $C_{16}H_{33}$, and $C_{18}H_{37}$ show a definite decrease in toxicity. The methyl compound, *e.g.*, is lethal for mice in doses of 0·05 g. per kg. (corresponding to 0·011 g. of arsenic per kg.), whereas a dose of the octadecyl compound of 0·8 g. per kg. is tolerated (0·105 g. of arsenic per kg.). The benzyl compound is about as toxic as the methyl derivative, 0·05 g. (0·0091 g. of arsenic) per kg. being lethal. No decrease in toxicity was observed when the 4-aminophenyl residue—in the 2-ethylhexyl compound ($CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CHEt{\cdot}CH_2{\cdot}$)—was replaced by the β-amino-α-naphthyl radical (see Part I, where these two 2-ethylhexyl compounds and the cetyl representative of the above series are described). In view of the low toxicity of the higher members, the octadecyl compound was tested against various strains of trypanosomes : it proved slightly effective.

EXPERIMENTAL.

Alkylanilines.—The methyl, dimethyl, ethyl, and benzyl compounds were commercial samples; the others were prepared as follows : Aniline (3 mols.) and the appropriate alkyl bromide (1 mol.) were heated for 3 hours on the water-bath, the mass poured into alkali solution, the organic layer washed with water, and the alkylaniline isolated by fractional vacuum distillation. The new members of the series are listed in Table I; the *n*-amyl, *iso*amyl, *n*-hexyl, and *n*-heptyl compounds have been described by Hickinbottom (J., 1932, 2396; 1937, 1119), and the cholesteryl compound by Lieb *et al.* (*Annalen*, 1935, **509**, 222).

TABLE I.

Alkylanilines, $R{\cdot}NH{\cdot}C_6H_5$.

R.	B. p.	$n_D^{26°}$.	Formula.	Found, %. C.	Found, %. H.	Required, %. C.	Required, %. H.
sec.-*Butyl*	225°/759 mm.; 112—114°/22 mm.	1·5318	$C_{10}H_{15}N$	79·7	10·3	80·5	10·0
sec.-*Butylcarbinyl*	236°/758 mm.; 142°/25 mm.		$C_{11}H_{17}N$	81·5	9·8	81·0	10·4
2-*Methylpentyl*	138°/22 mm.	1·5241	$C_{12}H_{19}N$	81·2	10·7	81·3	10·7
Dodecyl	140°/0·2 mm.; m. p. 28°		$C_{18}H_{31}N$	82·5	11·3	82·7	11·9
Tetradecyl	180°/4 mm.; m. p. 42° *		$C_{20}H_{35}N$	82·4	12·0	83·0	12·1
Octadecyl	196°/0·6 mm.; m. p. 42°		$C_{24}H_{43}N$	83·6	12·2	83·5	12·5

* Crystallised from methanol. Myristylaniline was characterised as 3 : 5-dinitrobenzomyristylanilide (from light petroleum), m. p. 83°.

4-Alkylaminoazobenzene-4'-arsonic Acids.—A solution of atoxyl (11·9 g.) in a mixture of concentrated sulphuric acid (4·8 c.c.) and water (125 c.c.) was diazotised with sodium nitrite (3·5 g.) and added to the equivalent amount of the substituted aniline, dissolved in glacial acetic acid (four times its weight). The product was collected after 24 hours' standing at room temperature and purified by trituration with and crystallisation from a suitable solvent. The methyl compound has been prepared by Jacobs and Heidelberger (*J. Amer. Chem. Soc.*, 1921, **43**, 1632). Most of the dyes had no definite m. p., but they all crystallised well (Table II).

Toxicity Tests.—These were carried out with white mice (Table III). Only the butyl and the *sec.*-butyl compound have been tested on rats.

The Trypanocidal Properties of 4-Octadecylaminoazobenzene-4'-arsonic Acid (*W* 47).—A solution of the acid in the equivalent amount of aqueous sodium hydroxide was injected intraperitoneally. In Table IV its activity is compared with that of the minimum curative doses of atoxyl.

TABLE II.

4-Alkylaminoazobenzene-4′-arsonic acids, $R{\cdot}NH{\cdot}C_6H_4{\cdot}N{:}N{\cdot}C_6H_4{\cdot}AsO_3H_2$.

R.	Formula.	Found, %. C.	Found, %. H.	Required, %. C.	Required, %. H.	Remarks.
[Methyl	$C_{13}H_{14}O_3N_3As$]	46·3	4·1	46·6	4·2	
Dimethyl	$C_{14}H_{16}O_3N_3As$	48·7	4·3	48·1	4·6	From aqueous formic acid; m. p. 310° (decomp.)
Ethyl	$C_{14}H_{16}O_3N_3As$	48·2	5·0	48·1	4·6	From aqueous alcohol; m. p. 276° (decomp.)
n-*Propyl*	$C_{15}H_{18}O_3N_3As$	48·9	5·3	49·6	5·0	From glacial acetic acid; m. p. 286° (decomp.)
n-*Butyl*	$C_{16}H_{20}O_3N_3As$	50·4	5·5	51·0	5·3	From alcohol
iso*Butyl*	$C_{16}H_{20}O_3N_3As$	50·4	5·3 (N, 11·8)	51·0	5·3 (N, 11·1)	From 70% methanol; brick-red; m. p. 303° (decomp.)
sec.-*Butyl*	$C_{16}H_{20}O_3N_3As$	50·8	5·0	51·0	5·3	From alcohol; contains 1 mol. of alcohol of crystallisation
n-*Amyl*	$C_{17}H_{22}O_3N_3As$	52·1	5·1	52·2	5·6	From aqueous alcohol or glacial acetic acid
iso*Amyl*	$C_{17}H_{22}O_3N_3As$	53·0	5·0	52·2	5·6	From 80% alcohol; resembles chromic acid in appearance
sec.-*Butylcarbinyl*	$C_{17}H_{22}O_3N_3As$	51·8	5·6	52·2	5·6	Needles from glacial acetic acid; m. p. 245° (decomp.)
n-*Hexyl*	$C_{18}H_{24}O_3N_3As$	53·0	6·8	53·3	5·9	From 50% acetic acid; m. p. 270° (decomp.)
2-*Methylpentyl*	$C_{18}H_{24}O_3N_3As$	52·8	5·5	53·3	5·9	M. p. 265° (decomp.)
n-*Heptyl*	$C_{19}H_{26}O_3N_3As$	55·2	6·5	54·4	6·2	From alcohol
n-*Dodecyl*	$C_{24}H_{36}O_3N_3As$	60·0	7·3	58·9	7·4	From 80% acetic acid, brick-red
n-*Tetradecyl*	$C_{26}H_{40}O_3N_3As$	61·5	8·2	60·3	7·7	From alcohol; brown-red plates
n-*Octadecyl*	$C_{30}H_{48}O_3N_3As$	62·3	8·1	62·9	8·4	From alcohol; orange leaflets
cyclo*Hexyl*	$C_{18}H_{22}O_3N_3As$	53·7	5·7	53·6	5·4	From glacial acetic acid or formic acid; brown-red; m. p 292° (decomp.)
Benzyl	$C_{19}H_{18}O_3N_3As$	55·4	4·2	55·5	4·4	From glacial acetic acid and alcohol; * brown; m. p. 340° (decomp.)
Cholesteryl	$C_{39}H_{56}O_3N_3As$	67·3	8·3	67·9	8·1	From glacial acetic acid and alcohol; † dark red; m. p. 237° (decomp.)

* Can be purified through the sodium salt, which is precipitated from sodium carbonate solution in brown crystals.

† The dye is soluble in benzene.

TABLE III.

Toxicity tests.

R.	Max. dose tolerated (g. per kg.).	Min. lethal dose (g. per kg.).	R.	Max. dose tolerated (g. per kg.).	Min. lethal dose (g. per kg.).
H	0·01	0·02	2-Methylpentyl	0·02	0·04
Methyl	0·025	0·05	Heptyl	0·05	0·1
Dimethyl	0·04	0·05	2-Ethylhexyl	0·04	0·05
Ethyl	0·01	0·02	Dodecyl	0·3	0·4
Propyl	0·02	0·04	Tetradecyl	0·5	0·6
Butyl	0·01	0·02	Hexadecyl (cetyl)	0·7	0·8
*iso*Butyl	0·02	0·04	Octadecyl	0·8	0·9
sec.-Butyl	0·01	0·02	Benzyl	0·025	0·05
n-Amyl	0·01	0·02	*cyclo*Hexyl	0·01	0·02
*iso*Amyl	0·05	0·075	1-(4′-Arsonobenzeneazo)-2-(β-ethylhexylamino)-naphthalene	0·04	0·05
sec.-Butylcarbinyl	0·03	0·04			
n-Hexyl	0·05	0·1			

TABLE IV.

W 47, dose (g. per kg.).	*Trypanosoma brucei.**	*Trypanosoma equiperdum.*
0·4	No effect	Effective; relapse after 9 days
0·5	Effective, but relapse after about 15 days	—
0·6	—	Effective; relapse after 11 days
0·7	Effective, but relapse after 30 days	Effective; relapse after 18 days
atoxyl	0·2 g.; cure	0·15 g.; cure

* This strain, which was obtained from the Liverpool School of Tropical Medicine, is sensitive to arsenicals.

With W 47 the parasites disappear from the blood only after a relatively long time. The trypanosomes disappear within 12 hours after injection of atoxyl, whereas the above azo-dye gives the same effect only after 48—96 hours. We ascribe this fact to the low reducibility of the octadecylamino-compound, as compared with that of atoxyl (compare Breyer, *Biochem. Z.*, 1939, **301**, 65). Therefore the inactivity of small doses is probably due to the active form of the dye not reaching the required concentration in time, and it may be expected that these in themselves inactive doses would produce an effect when combined with subcurative doses of a "normal," *i.e.*, quickly reducible, arsenical, such as atoxyl. Table V shows that this conclusion is justified. In the experiments listed, 4-octadecylaminoazobenzene-4'-arsonic acid (W 47) was combined with *N*-dichloroacetylarsanilic acid, a new arsenical to be described in a forthcoming paper (W 129), which is active against *Trypanosoma brucei* (*equiperdum*) in doses of 0·1 (0·05) g. per kg. and clears the blood from parasites for 3 days in doses of 0·025 (0·01) g. per kg.

TABLE V.

Substance.	Dose (g. per kg.).	*Trypanosoma brucei.*
W 129	0·025	Relapse after 3 days
W 47	0·2	No action
W 47	0·3	No action
W 47	0·4	Prolonged duration of the illness (by about 2 days)
W 129+W 47	0·025+0·2	Relapse after 7 days
W 129+W 47	0·025+0·3	Relapse after 10 days
W 129+W 47	0·025+0·4	Relapse after 21 days
		Trypanosoma equiperdum.
W 129	0·01	Relapse after 2 days
W 47	0·2	No action
W 129+W 47	0·01+0·2	Relapse after 8 days

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