850. *Bisdiguanides having Antibacterial Activity.*

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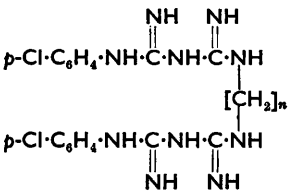
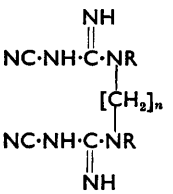
A series of bisdiguanides has been prepared either by condensing one mol. of a biscyanoguanidine with two mols. of an amine hydrochloride, or by the converse interaction of two mols. of an *N*-arylcyanoguanidine with one of a diamine salt. One of the products, 1 : 6-di-(N^5-*p*-chlorophenyl-N^1-diguanido)hexane (chlorohexidine B.P.C.), has found practical use as an antibacterial agent.

The occurrence of antimalarial activity in certain substituted diguanides [1] stimulated the search for other therapeutically useful members of this series, and in due course led to the discovery of high antibacterial activity, more especially amongst a series of bisdiguanides. One such compound, 1:6-di-(N^5-*p*-chlorophenyl-N^1-diguanido)hexane (I; $n = 6$) has recently been introduced into medical and veterinary practice under the common name chlorhexidine B.P.C. ("Hibitane" *).[2] The present communication is concerned with the chemistry of this substance, and also with that of related compounds, many of which were prepared during the investigation of its mode of action.

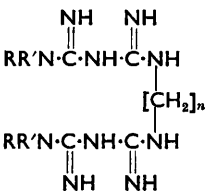
The original observation of marked bacteriostatic action was made with the mixture of polymeric diguanides that resulted from the fusion of 1 : 6-di-(N^3-cyano-N^1-guanidino)-hexane (II; R = H, $n = 6$) with hexamethylenediamine dihydrochloride. Attempts to



(I)



(II)



(III)

determine actual chain lengths, or to separate the mixture into homogeneous fractions, were unsuccessful. Since, however, it was known that molecules carrying only one diguanide residue were but weakly antibacterial, it was clearly desirable to determine the degree of molecular complexity necessary for high antimicrobial potency. For this purpose the step-wise synthesis of polydiguanides was undertaken. In the event, full biological activity was reached immediately two diguanide systems were incorporated into each drug molecule, and effort was then concentrated on ascertaining the optimum distance which should exist between these two residues and the most effective types of end groupings—whether, for example, antibacterial activity was highest in bisdiguanides in which the terminal groups were aryl, alkyl, or heterocyclic. In addition it was desired to examine the effect of replacing the diguanide residues by other less complex basic groups such as that of guanidine. The relevant biological findings have already been reported elsewhere;[3] briefly it was found that highest antibacterial activity occurred in the series (I), and in particular when $n = 5$, 6, or 7. Since hexamethylenediamine was most readily available to us, the majority of the subsequent preparations were based on this substance. The introduction of hydroxyl or carbonyl substituents into the terminal aryl groups almost eliminated antibacterial action, as did the replacement of diguanide by guanidine, while the analogous wholly alkyl-bisdiguanides (III; R = alkyl, R′ = H or alkyl, $n = 6$) were only fractionally as effective (one-third to one-tenth) as the compound (I).

The standard methods of preparing the bisdiguanides were employed, similar to those developed for the simple antimalarial monodiguanides.[1,4] The first required the reaction

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[1] Curd and Rose, *J.*, 1946, 729.
[2] B.P. 705,838.
[3] Davies, Francis, Martin, Rose, and Swain, *Brit. J. Pharmacol.*, 1954, **9**, 192.
[4] Curd, Hendry, Kenny, Murray, and Rose, *J.*, 1948, 1630.

TABLE 1. *Biscyanoguanidines* (II) (R = H except as stated).

–[CH$_2$]$_n$–	Butanol (c.c.)/ 0·01 mol.	Reaction time (hr.)	Solvent for crystn.	M. p.†	Yield (%)	Formula	Found (%) C	H	N	Required (%) C	H	N
[CH$_2$]$_2$	15	12	H_2O	248—250° ‡	57	$C_6H_{10}N_8$	36·35	5·3	56·8	37·1	5·15	57·7
[CH$_2$]$_3$	15	16	,,	206 ‡	50—60	$C_7H_{12}N_8$	39·95	5·9	53·5	40·4	5·8	53·5
[CH$_2$]$_4$	14	18	,,	197—199	74	$C_8H_{14}N_8$	43·0	6·9	48·1	43·2	6·3	50·4
[CH$_2$]$_5$	14	20	,,	169—171	58	$C_9H_{16}N_8$	45·2	6·3	46·6	45·8	6·8	47·45
[CH$_2$]$_6$	14	8	,,	202—203 (s 199)	70—80	$C_{10}H_{18}N_8$	47·45	7·45	44·4	48·0	7·2	44·8
[CH$_2$]$_7$	12·5	16	Aq. EtOH	144—146 (s 138—140)	30—40	$C_{11}H_{20}N_8,\frac{1}{2}H_2O$	48·25	7·55	41·25	48·3	7·7	41·0
[CH$_2$]$_{10}$	15	,,	*Aq. EtO·C_2H_4·OH	184—186 (s 182)	v. low	$C_{14}H_{26}N_8$	54·15	8·0	36·6	54·9	8·5	36·6
[CH$_2$]$_6$ §	15	,,	* Aq. EtOH	186—187	83	$C_{18}H_{34}N_8$	59·3	9·45	30·85	59·7	9·4	30·9
[CH$_2$]$_3$·O·[CH$_3$]$_3$	10	,,	* H_2O	138—140 (s 136)	56	$C_{10}H_{18}ON_8$	44·45	6·65	42·3	45·1	6·8	42·1
[CH$_2$]$_3$·O·C_6H_4(*p*)·O·[CH$_2$]$_3$	15	15	*Aq. EtO·C_2H_4·OH	204—206	56	$C_{16}H_{22}O_2N_8$	53·25	6·35	30·1	53·6	6·1	31·3
[CH$_2$]$_3$·O·[CH$_2$]$_2$·O·[CH$_2$]$_3$	10	16	Gum used without further purification after removal of salt and solvent.									

* Prisms. † s = sinters. ‡ With decomp. § R = Bu^t.

TABLE 2. *Bis*-p-*chlorophenyldiguanidines* (I).

Code no.	–[CH$_2$]$_n$–	2-Ethoxyethanol (c.c.) per 0·01 mol.	Reaction time (hr.)	Solvent for crystn.	M. p.	Yield (%)	Formula	Found (%) C	H	N	Cl	Required (%) C	H	N	Cl
20,184	[CH$_2$]$_2$ [a]	14	6	H_2O	245—246° *	26	$C_{18}H_{22}N_{10}Cl_2,2HCl,H_2O$	40·2	5·2	26·3	25·9	40·0	4·8	25·9	26·3
12,483	[CH$_2$]$_3$ [b]	20	4	,,	224—226 *	50	$C_{19}H_{24}N_{10}Cl_2,2HCl,H_2O$	41·4	5·3	24·5	25·2	41·15	5·05	25·3	25·6
12,484	[CH$_2$]$_4$ [c]	20	3	,,	253—254 *	59	$C_{20}H_{26}N_{10}Cl_2,2HCl$	43·7	5·2	24·6	25·2	43·6	5·1	25·5	25·8
12,485	[CH$_2$]$_5$ [b]	20	6	,,	250	58	$C_{21}H_{28}N_{10}Cl_2,2HCl$	44·4	5·2	24·2	25·5	44·7	5·3	24·8	25·2
10,040	[CH$_2$]$_6$	See text													
12,486	[CH$_2$]$_7$	25	1½	,,	253—254	57	$C_{23}H_{32}N_{10}Cl_2,2HCl$	46·0	5·3	23·7	25·0	46·6	5·7	23·6	24·0
11,383	[CH$_2$]$_{10}$	20	6	Aq. EtOH	246—248	60	$C_{26}H_{38}N_{10}Cl_2,2HCl$	49·25	6·3	21·4	—	49·2	6·3	22·1	—
14,345	[CH$_2$]$_3$·O·[CH$_2$]$_3$	18	2	H_2O	236—238	73	$C_{22}H_{30}ON_{10}Cl_2,2HCl$	44·4	5·5	23·3	24·0	44·4	5·4	23·6	24·0
14,411	[CH$_2$]$_3$·O·[CH$_2$]$_2$·O·[CH$_2$]$_3$	20	2½	Aq. $COMe_2$	204—205	50	$C_{24}H_{34}O_2N_{10}Cl_2,2HCl$	46·7	5·4	22·6	22·2	45·1	5·6	22·0	22·3
11,382	[CH$_2$]$_3$·O·C_6H_4(*p*)·O[CH$_2$]$_3$	20	9	Aq. EtOH	248—249	35	$C_{28}H_{34}O_2N_{10}Cl_2,2HCl$	49·05	5·2	20·4	—	49·0	5·3	20·4	—
11,384	C_6H_4(*p*) †	—	—	,,	253—254	70	$C_{22}H_{22}N_{10}Cl_2,2HCl$	46·15	4·35	24·7	—	46·3	4·2	24·6	—
11,385	(*p*)C_6H_4·CH_2·C_6H_4(*p*) †	—	—	,,	—	50	$C_{29}H_{28}N_{10}Cl_2,2HCl$	52·85	4·6	20·4	—	52·7	4·5	21·2	—

* With decomp. † Method (ii), see text.

TABLE 3. 1 : 6-*Bisdiguanidohexanes* (III; R′ = H, *n* = 6).

Code no.	R	2-Ethoxyethanol (c.c.)/0·01 mol.	Reaction time (hr.)	Solvent	M. p.	Yield (%)	Formula	Found (%) C	H	N	Cl	Required (%) C	H	N	Cl
10,387	Ph [b]	16	3	H_2O	242—244°	74	$C_{22}H_{32}N_{10},2HCl$	51·55	6·7	27·4	12·9	51·9	6·7	27·5	13·95
10,688	*p*-C_6H_4Me	25	2	Aq. EtO·C_2H_4·OH	263—264	90	$C_{24}H_{36}N_{10},2HCl$	53·45	6·5	25·65	—	53·6	7·1	26·1	—
11,108	*p*-C_6H_4·OH [c]	,,	2½	EtOH–PrOH	200—202	40	$C_{22}H_{32}O_2N_{10},2HCl$	48·4	6·15	25·3	—	48·8	6·3	25·9	—
10,689	*p*-C_6H_4·OMe [b]	20	2	MeOH–EtOH	238—240	78	$C_{24}H_{36}O_2N_{10},2HCl$	49·85	6·5	25·5	—	50·6	6·7	24·6	—
10,691	*p*-C_6H_4·CO_2H	25	6	H_2O	218—224 *	42	$C_{24}H_{32}O_4N_{10},2HCl$	47·65	6·1	22·7	—	48·2	5·7	23·4	—
10,040	*p*-C_6H_4Cl [c]	25	3	,,	260—262	90	$C_{22}H_{30}N_{10}Cl_2,2HCl$	45·5	5·8	24·25	23·8	45·7	5·5	24·2	24·9
11,380	*m*-C_6H_4Cl	25	9	Aq. EtOH	233—234	10	$C_{22}H_{30}N_{10}Cl_2,2HCl$	46·3	5·95	23·95	—	45·7	4·5	24·2	—
11,386	3 : 4-$C_6H_3Cl_2$	13	9	EtO·C_2H_4·OH	259—260	68	$C_{22}H_{28}N_{10}Cl_4,2HCl$	41·2	5·0	21·5	—	40·8	4·6	21·6	—
11,381	2 : 5-$C_6H_3Cl_2$ [b]	25	8	Aq. EtOH	249—250	34	$C_{22}H_{28}N_{10}Cl_4,2HCl$	40·6	5·2	20·9	—	40·8	4·6	21·6	—
11,110	α-$C_{10}H_7$	,,	8	Aq. AcOH	257—258	48	$C_{30}H_{36}N_{10},2HCl$	58·9	6·1	22·7	—	59·1	5·9	23·0	—
10,388	β-$C_{10}H_7$ [b]	,,	3	H_2O	252—254	74	$C_{30}H_{36}N_{10},2HCl$	59·5	6·2	23·1	10·9	59·1	5·9	23·0	11·7

[a] Prisms. [b] Needles. [c] Plates. * With decomp.

in nitrobenzene of an *N*-phenylcyanoguanidine with each of the amino-groups of a diamine, and the second, more generally used, was the complementary process in which the biscyanoguanidine (II) interacted with two molecular proportions of the amine NHRR′ (see III), preferably in boiling 2-ethoxyethanol. The biscyanoguanidines were themselves readily accessible from the reaction of the appropriate diamine dihydrochloride with two molecular proportions of sodium dicyanimide in boiling butanol. The yield of the crystallised products was 50—80%, that of the hexamethylene derivative being in the upper range. The intermediate preparations also included examples in which the nitrogen atoms adjacent to the polymethylene chain carried an alkyl group (II; R = alkyl), and others in which ether-oxygen atoms, and in one case additionally a *p*-substituted phenyl residue, were interposed in the polymethylene chain (see Table 1).

A slightly modified procedure was employed for the production of the bisdiguanides (III; R and/or R′ = alkyl) in that a solvent was not required, and the usual practice was to fuse an intimate mixture of the biscyanoguanidines with the alkylamine hydrochlorides at bath temperatures in the range 150—160°. Two bisdiguanides were also made in which the terminal groups were derived from 8-amino-6-methoxyquinoline and 2 : 5-diamino-4 : 6-dimethylpyrimidine severally: in these instances, 2-ethoxyethanol was used as solvent. Finally, the bisguanidine corresponding to the bisdiguanide (I; $n = 6$) was prepared from the related thiourea, itself formed by the interaction of hexamethylene di*iso*thiocyanate with *p*-chloroaniline in boiling ethanol. Amination was effected by alcoholic ammonia in the presence of mercuric oxide.

Experimental

General Method of Preparation of Biscyanoguanidines.—The diamine dihydrochloride (1·0 mol.) and sodium dicyanimide (2·0 mols.) were powdered together, mixed with butanol, and stirred and heated under reflux (bath-temp. 130—140°) for 8—16 hr. After cooling, either the insoluble solid was filtered off and washed with cold water to remove sodium chloride, or, where the product remained in solution, the sodium chloride was first filtered off and the filtrate was then evaporated to dryness under reduced pressure. Details of the experiments are given in Table 1.

1 : 6-*Di*-(N^5-p-*chlorophenyl*-N^1-*diguanido*)*hexane Dihydrochloride* (10,040).—(i) 1 : 6-Di-(*N*^{3}-cyano-*N*^{1}-guanidino)hexane (30 g., 0·12 mol.), *p*-chloroaniline hydrochloride (39·6 g., 0·12 mol.), and 2-ethoxyethanol (300 c.c.) were stirred together under reflux (bath-temp. 130—140°) for 3 hr. After cooling, the microcrystalline *dihydrochloride* [62 g., 90%; m. p. 260—262° (decomp.)] was filtered off and washed with ethanol. Recrystallisation from water, in which it is sparingly soluble, gave plates, m. p. unchanged (Found: C, 45·5; H, 5·8; N, 24·25; Cl, 23·8. $C_{22}H_{30}N_{10}Cl_2,2HCl$ requires C, 45·7; H, 5·5; N, 24·2; Cl, 24·9%). The *base* could be obtained sensibly free from the hydrochloride only by adding a solution of the latter in hot water to an excess of aqueous sodium hydroxide, and not by the reverse procedure. It formed colourless needles (from methanol), m. p. 133·5—134° (corr.) (Found: C, 52·1; H, 6·0; N, 27·7. $C_{22}H_{30}N_{10}Cl_2$ requires C, 52·3; H, 5·9; N, 27·7%). The *diacetate* crystallised on cooling from a solution of the base in hot dilute acetic acid and after recrystallisation from water formed prisms, m. p. 154—155° (corr.) (Found: C, 48·6; H, 6·4; N, 21·7; loss at 120° *in vacuo*, 3. $C_{22}H_{30}N_{10}Cl_2,2C_2H_4O_2,H_2O$ requires C, 48·5; H, 6·2; N, 21·75; H_2O, 2·8%).

(ii) *N*^{3}-*p*-Chlorophenyl-*N*^{1}-cyanoguanidine (19·5 g., 0·1 mol.), hexamethylenediamine dihydrochloride (10·4 g., 0·11 mol.), and nitrobenzene (50 c.c.) were stirred together at 140—145° (bath-temp.) for 16 hr. The suspension was filtered hot and the well-pressed filter cake was suspended in ethanol and stirred for 1½ hr. Refiltration gave the crude dihydrochloride (15·3 g., 53%; m. p. 257—258°). Substitution of methyl*cyclo*hexanol for nitrobenzene gave only a 20% yield.

All the bis-*p*-chlorophenyldiguanides listed in Tables 2 and 3, with two exceptions, were prepared by method (i). The two exceptions, 11,384 and 11,385 (Table 2), were prepared by method (ii).

Miscellaneous Bisdiguanides.—1 : 6-*Di*-(N^5-n-*butyl*-N^1-*diguanido*)*hexane dihydrochloride* (9458). 1 : 6-Di-(*N*^{3}-cyano-*N*^{1}-guanidino)hexane (1·25 g., 0·055 mol.) and *n*-butylamine hydrochloride (1·1 g., 0·01 mol.) were powdered together and heated at 150—155° for 2 hr. The clear melt solidified after 1½ hr., and the *product* was obtained as a microcrystalline solid

(1·4 g., 59·6%; m. p. 223—224° with sintering at 190—192°) by addition of ethyl acetate to a solution in ethyl alcohol (Found: C, 45·5; H, 8·55; N, 29·55; Cl, 15·15. $C_{18}H_{40}N_{10},2HCl$ requires C, 46·1; H, 8·95; N, 29·85; Cl, 15·1%). The salt was freely soluble in water.

1 : 6-*Di*-(N⁵-iso*propyl*-N¹-*diguanido*)*hexane dihydrochloride* (9357). 1 : 6-Di-(*N*⁵-cyano-*N*³-guanidino)hexane (1·25 g., 0·005 mol.) and *iso*propylamine hydrochloride (0·9 g., 0·01 mol.) were intimately mixed and heated together at 160° for ½ hr. Similarly crystallised from alcohol–ethyl acetate, the microcrystalline *dihydrochloride* (1·1 g., 50%) had m. p. 256—257° (decomp.) (Found: C, 43·3; H, 8·3; N, 32·3. $C_{16}H_{36}N_{10},2HCl$ requires C, 43·5; H, 8·6; N, 31·75%) and was freely soluble in water.

1 : 6-*Di*-(N⁵-cyclo*hexyl*-N¹-*diguanido*)*hexane dihydrochloride* (9382). 1 : 6-Di-(*N*³-cyano-*N*¹-guanidino)hexane (1·25 g., 0·005 mol.) and *cyclo*hexylamine hydrochloride (1·35 g., 0·01 mol.) were intimately mixed and heated at 160° for 1 hr. Similarly recrystallised from alcohol–ethyl acetate, the *dihydrochloride* had m. p. 234—236° after sintering at 225—230° (Found: N, 26·25. $C_{22}H_{44}N_{10},2HCl$ requires N, 26·9%).

1 : 6-*Di*-(N⁵N⁵-*pentamethylene*-N¹-*diguanido*)*hexane dihydrochloride* (9383). 1 : 6-Di-(*N*³-cyano-*N*¹-guanidino)hexane (1·25 g., 0·005 mol.) and piperidine hydrochloride (1·2 g., 0·01 mol.) were intimately mixed and heated at 150° for 1½ hr. The originally clear melt solidified after 1 hr. Similarly recrystallised from ethanol–ethyl acetate, the *dihydrochloride* formed prisms, m. p. 231° (1·1 g., 45%) (Found: N, 28·6. $C_{20}H_{40}N_{10},2HCl$ requires N, 28·4%).

1 : 6-*Di*-[N⁵-(2-*amino*-4 : 6-*dimethylpyrimidin*-5-*yl*)-N¹-*diguanido*]*hexane disulphate* (10,160). 2-Amino-4 : 6-dimethylpyrimidine (5·6 g., 0·04 mol.) was dissolved in 2-ethoxyethanol (50 c.c.) and 1 : 6-di-(*N*³-cyano-*N*¹-guanidino)hexane (5·0 g., 0·02 mol.) added to the stirred solution, followed by ethanolic hydrochloric acid (4·8N; 8·5 c.c., 2 equivs.). The mixture was stirred under reflux for 68 hr., the 2-ethoxyethanol removed under reduced pressure, and the residue dissolved in water (50 c.c.). The brown gum precipitated by addition of 10N-sodium hydroxide was washed by decantation with water and redissolved in dilute hydrochloric acid; the solution was made alkaline with ammonia and filtered (charcoal), and the base reprecipitated with sodium hydroxide solution. After being further washed with water by decantation, the still gummy base was converted into the *disulphate* (7 g.) by adding successively sulphuric acid (*d* 1·84; 3 c.c.) and diethylamine to alkalinity, to its solution in 1 : 1 aqueous ethanol (100 c.c.), filtering (charcoal), and finally adding excess of dry ethanol. Recrystallisation from water afforded prisms (1·8 g.), m. p. 220—225° (decomp.) (Found: C, 35·05; H, 6·4; N, 29·15; S, 8·2. $C_{22}H_{38}N_{16},2H_2SO_4,2H_2O$ requires C, 34·8; H, 6·1; N, 29·5; S, 8·4%).

1 : 6-*Di*-[N⁵-(6-*methoxyquinolin*-8-*yl*)-N¹-*diguanido*]*hexane dihydrochloride* (10,690). 8-Amino-6-methoxyquinoline (7·0 g., 0·04 mol.), 1 : 6-di-(*N*³-cyano-*N*¹-guanidino)hexane (5·0 g., 0·02 mol.), 2-ethoxyethanol (50 c.c.), and ethanolic hydrochloric acid (6·2N; 6·5 c.c., 2 equivs.) were stirred together under reflux for 3 hr. The orange-coloured suspension slowly dissolved to a clear solution, which finally deposited crystals of the *dihydrochloride* (5·1 g., 45%; m. p. 246—248°). This recrystallised from water in colourless felted needles, m. p. 246—248° (Found: C, 53·2; H, 5·95; N, 24·8. $C_{30}H_{38}O_2N_{12},2HCl$ requires C, 53·65; H, 5·95; N, 25·0%).

1 : 6-*Di*-(N³-p-*chlorophenyl*-N¹-*thioureido*)*hexane* (11,368). A mixture of hexamethylene di*iso*thiocyanate (10·0 g., 0·05 mol.), *p*-chloroaniline (13·0 g., 0·05 mol.), and ethanol (150 c.c.) was refluxed for 16 hr. and water was then added to crystallisation point. Cooling gave the crude *thiourea* (16 g.) which recrystallised from ethanol in colourless needles (13·8 g., 60%; m. p. 171—172°) (Found: C, 53·1; H, 5·25; N, 12·55. $C_{20}H_{24}N_4S_2Cl_2$ requires C, 52·75; H, 5·3; N, 12·3%).

1 : 6-*Di*-(N³-p-*chlorophenyl*-N³-*guanido*)*hexane* (11,717). Ammonia in ethanol (5·6N; 150 c.c.), followed by mercuric oxide (4·5 g.), was added to a cooled solution of the above thiourea (9·1 g.) in 2-ethoxyethanol (80 c.c.). The suspension was kept at 30—35° for 40 hr., then warmed to 60° and filtered. The bulk of the solvent was distilled under reduced pressure and the *guanidine* (3·5 g.; m. p. 146—147°) was precipitated by slow addition of water. It crystallised from ethanol–water in colourless needles (2·6 g.; m. p. 160—161°) (Found: C, 57·0; H, 6·25; N, 19·8. $C_{20}H_{26}N_6Cl_2$ requires C, 57·0; H, 6·2; N, 19·95%).

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[*Received, June 19th*, 1956.]