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DERIVATIVES OF 3 : 4-XYLIDINE AND RELATED COMPOUNDS AS INHIBITORS OF INFLUENZA VIRUS : RELATIONSHIPS BETWEEN CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY

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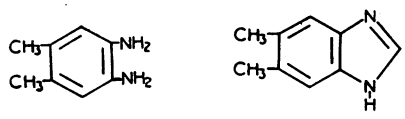
A series of compounds, based primarily on 3 : 4-xylidine, was examined for inhibitory activity towards the growth of influenza virus in tissue culture. Marked dependence of inhibitory activity upon chemical structure was observed particularly when the 3 : 4-xylyl group was replaced by other simple aryl radicals. *N*-(2-Piperidinoethyl)-3 : 4-xylidine dihydrochloride, a typical compound combining high intrinsic inhibitory activity with no obvious toxicity towards the host tissues, did not inactivate the virus directly before its adsorption, did not interfere with adsorption of virus by the tissues, and did not inhibit the release of freshly synthesized virus by the tissues, but specifically depressed the synthesis of viral haemagglutinin to a greater extent than it depressed the synthesis of complement-fixing soluble antigen. The inhibition of growth of influenza virus caused by this compound in tissue culture was reversed by appropriate addition of 4 : 5-dimethyl-*o*-phenylenediamine, but not apparently by riboflavin or by vitamin B_{12}. The action of this substance, and, by inference, of related compounds, in inhibiting viral synthesis may be the result of depressed cytoplasmic protein synthesis.

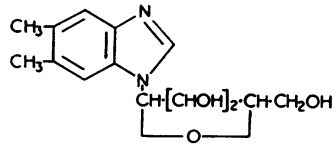
The effective chemotherapy of virus infections must combine inhibition of growth of the virus and lack of toxicity to the host, and the intimate dependence of the virus upon the metabolism of the susceptible host cell introduces difficulties which are absent from the chemotherapy of infections due to free-living bacterial and protozoal pathogens which possess active and unique metabolic patterns of their own. Both qualitative and quantitative differences may, however, exist between the biosynthetic requirements of virus and host cell, and these differences, if they can be detected, may allow the selective inhibition of virus multiplication by appropriate compounds. Although numerous substances are now known to influence the growth of influenza virus in tissue cultures, few definite pointers towards the effective chemotherapy of influenza have emerged. Structure/activity relationships, however, are more readily detectable in such cultures which are capable of providing useful information concerning the metabolic requirements for viral growth, or conditions for viral inhibition, in a cellular environment. The present communication describes a series of compounds which appeared to affect the growth of influenza virus in tissue culture in doses which were almost always without a gross toxic effect on the host tissue.

We decided to examine *N*-acyl derivatives of 4 : 5-dimethyl-*o*-phenylenediamine (I) bearing in the acyl radical basic groups capable of conferring solubility in water on the resulting compounds through the formation of neutral soluble salts as there were indications that this amine and related compounds had some significant biological activities in addition to its incorporation into riboflavin and into vitamin B_{12}. For example, it had been shown that the acid degradation product of vitamin B_{12}, 5 : 6-dimethylbenzimidazole (II) and its precursor (I) were without vitamin activity for exacting lactobacilli and were in fact toxic to these organisms (Hendlin and Soars, 1951), although 4 : 5-dichloro-*o*-phenylenediamine was an inhibitor of growth of various bacteria and the inhibitory effects were reversed by (I) (Woolley and Pringle, 1952). It was also shown (Tamm, Folkers, Shunk, and Horsfall, 1954) that 5 : 6-dimethylbenzimidazole (II) was inhibitory towards influenza virus although 5 : 6-dimethyl-1-α-D-ribofuranosylbenzimidazole (III) (α-ribazole), a moiety incorporating (II) present in vitamin B_{12}, was not inhibitory under similar circumstances. Replace-



(I)

(II)



(III)

ment of the two methyl groups in (III) by chlorine atoms, however, gave a very active inhibitor of the growth of influenza virus (Tamm *et al.*, 1954), and a further increase in activity was found in 4:5:6(or 5:6:7)-trichloro-1-β-D-ribofuranosylbenzimidazole (Tamm, Folkers, and Shunk, 1956).

METHODS

Chemical Methods

General Methods for the Preparation of Halogenoacylarylamides

Two methods were used: (i) the appropriate amine and chloroacetyl (or α-chloropropionyl) chloride were heated together in boiling benzene for 2 hr., or (ii) the amine and chloroacetyl chloride were allowed to react in glacial acetic acid in the cold in presence of sodium acetate as described by Löfgren (1948).

The following compounds have not been described previously (the yield and method of preparation follow the name of the compound): *α-chloro-6-nitroaceto-3:4-xylidide*, 88%, (i), yellow needles from benzene, m.p. 150° (Found: C, 49.6; H, 4.7. $C_{10}H_{11}O_3N_2Cl$ requires C, 49.5; H, 4.6%); *α-chloro-3:4-dimethoxyacetanilide*, 81%, (i), colourless needles from aqueous ethanol, m.p. 133 to 135° (Found: C, 52.2; H, 5.1. $C_{10}H_{12}O_3NCl$ requires C, 52.3; H, 5.2%); *α:3:4-trichloroacetanilide*, 89%, (ii), colourless needles from aqueous ethanol, m.p. 105 to 107° (Found: C, 40.5; H, 2.5; N, 6.1. $C_8H_6ONCl_3$ requires C, 40.3; H, 2.5; N, 5.9%); *α-chloro-N-diphenyl-2-ylacetamide*, 92%, (ii), colourless rods from aqueous ethanol, m.p. 98 to 100° (Found: C, 68.4; H, 4.9. $C_{14}H_{12}ONCl$ requires C, 68.4; H, 4.9%); *α-chloro-N-diphenyl-4-ylacetamide*, 80%, (ii), colourless plates from methanol, m.p. 176 to 178° (Found: C, 68.5; H, 5.0%); *α:5-dichloroaceto-o-toluidide*, 82%, (ii), colourless plates from aqueous ethanol, m.p. 128 to 130° (Found: C, 49.5; H, 3.9. $C_9H_9ONCl_2$ requires C, 49.6; H, 4.2%); *α-chloropropiono-3:4-xylidide*, 88%, (i), colourless needles from aqueous ethanol, m.p. 139 to 140° (Found: C, 62.4; H, 6.4. $C_{11}H_{14}ONCl$ requires C, 62.4; H, 6.7%).

Table I gives the explanation of symbols used in subsequent Tables.

General Methods for the Preparation of Piperidino-, Pyrrolidin-1-yl, and Dialkylamino-acylarylamides and their Hydrochlorides

The appropriate halogenoacylarylamide was heated with 2 molecular proportions of the requisite amine in benzene for 5 hr. Precipitated amine hydrochloride was collected and the filtrate was taken to dryness. Basic material in the residue was recovered by solution in 3 N-HCl, filtration, and ether extraction, followed by basification with ammonia. The resulting free base was then either collected or extracted with ether. The hydrochlorides were prepared by passing hydrogen chloride into acetone solutions of the bases. Salts which separated were collected and more soluble ones were isolated by evaporation of the acetone; they were then recrystallized either from methanol/ethyl acetate or from ethanol/ether (Table II).

6-Amino-α-piperidinoaceto-3:4-xylidide (IV) *Dihydrochloride.* — 6-Nitro-α-piperidinoaceto-3:4-xylidide (93% yield) separated from *n*-propanol in yellow needles, m.p. 272 to 273° (Found: C, 62.1; H, 7.4. $C_{15}H_{21}O_3N_3$ requires C, 61.9; H, 7.3%). 8.1 g. of the substance was reduced in ethanol (200 ml.) in the presence of Raney nickel in hydrogen at room temperature and 3 atm.; the product was isolated in the usual way and converted into the dihydrochloride (Table II, 14).

α-Piperazin-1'-ylaceto-3:4-xylidide Hydrochloride. —α-Chloroaceto-3:4-xylidide (3.94 g.) and benzyloxycarbonylpiperazine (8.8 g.) were allowed to react in benzene in the manner described above. The intermediate α-(benzyloxycarbonylpiperazin-1-yl)-aceto-3:4-xylidide was a crystalline solid (5.35 g.), m.p. 177 to 180°. Removal of the benzyloxycarbonyl group was effected in ethanol solution at room temperature and atmospheric pressure in hydrogen in the presence of 5% palladized charcoal (14 hr.) (Table II, 28).

Glycyl-3:4-xylidide. — α-Chloroaceto-3:4-xylidide (19.8 g.) was allowed to react with saturated (0°) ethanolic ammonia (500 ml.), following the method described by Hill and Kelsey (1922) for the 4-chloro analogue, affording secondary amine (3.18 g.), m.p. 164 to 166°, and a main crop (13.4 g.) of the required primary amine (Table II, 32).

TABLE I

EXPLANATION OF SYMBOLS USED IN SUBSEQUENT TABLES TO SHOW EXTENT OF INHIBITORY ACTIVITY

Mean Log_2 Control Titre − Mean Log_2 Test Titre	Yield of Virus as % of Control	Inhibitory Activity
<1·0	⩾ 50	−
1·0–2·0	25–50	+
2·0–3·0	12·5–25	++
3·0–4·0	6·25–12·5	+++
>4·0	<6·25	++++

TABLE II

INHIBITORY ACTIVITY AND CERTAIN PHYSICAL AND CHEMICAL PROPERTIES OF PIPERIDINO-, PYRROLIDIN-1-YL, AND DIALKYLAMINO-ACYLARYLAMIDES AND THEIR HYDROCHLORIDES

Aryl·NH·CO·CHR·NR^{1}R^2

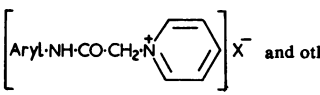
Symbols as in Table I. a = aqueous ethanol; b = ethanol/ether; c = methanol/ethyl acetate; d = ethyl acetate/petrol; e = benzene; f = benzene/petrol; g = needles; h = plates; k = Erdtman and Löfgren (1937) record base, m.p. 99 to 100°; l = Löfgren (1946) records base, m.p. 96 to 98°, hydrochloride, m.p. 171 to 172°; m = Löfgren and Lundquist (1946) record base, m.p. 115 to 116°, hydrochloride, m.p. 177 to 180°; n = Hall and Turner (1948); o = Löfgren (1946) records hydrochloride, m.p. 168 to 169°; t = toxic.

		Inhibitory Activity (0·1 mg./ml.)	Yield (%)	M.p. (Solvent; Cryst. Form)	Mol. Formula	Found (%)			Required (%)		
						C	H	N	C	H	N
1	α-Piperidinoacetanilide[k]		54	97–98° (ag)	$C_{13}H_{18}ON_2$	71·7	8·2	12·6	71·5	8·3	12·8
	hydrochloride	+	—	181–183° (hg)	$C_{13}H_{18}ON_2$,HCl	61·4	7·6	11·1	61·3	7·5	11·0
2	α-Piperidinoacetbenzylamide		—	43–46°							
	hydrochloride	++	66	148–150° (ch)	$C_{14}H_{20}ON_2$,HCl	62·7	7·6	10·1	62·5	7·9	10·4
3	α-Piperidinoaceto-*o*-toluidide[l]		77	95–98° (ah)	$C_{14}H_{20}ON_2$	72·5	8·7	11·9	72·4	8·7	12·1
	hydrochloride	−	—	168–171°							
4	α-Piperidinoaceto-*m*-toluidide		—								
	hydrochloride	+++	65	185–187° (cg)	$C_{14}H_{20}ON_2$,HCl	62·6	7·6	10·4	62·5	7·9	10·4
5	α-*Piperidinoaceto*-p-*toluidide*		85	65–67° (ag)	$C_{14}H_{20}ON_2$	72·3	8·4	11·9	72·4	8·7	12·1
	hydrochloride	++	—	212–214°							
6	α-Piperidinoaceto-2: 3-xylidide		84	73–75° (ag)							
	hydrochloride	+	—	221–223° (cg)	$C_{15}H_{22}ON_2$,HCl	63·6	8·0	10·0	63·7	8·2	9·9
7	α-*Piperidinoaceto*-2: 4-*xylidide*		77	86–88° (ag)	$C_{15}H_{22}ON_2$	73·0	9·0	11·2	73·2	9·0	11·4
	hydrochloride	−	—	217–219°							
8	α-*Piperidinoaceto*-2: 5-*xylidide*		83	95–96° (ah)	$C_{15}H_{22}ON_2$	73·0	8·7	11·4	73·2	9·0	11·4
	hydrochloride	++	—	185–187°							
9	α-Piperidinoaceto-2: 6-xylidide[m]		79	112–114° (ag)	$C_{15}H_{22}ON_2$	73·0	8·9	11·3	73·2	9·0	11·4
	hydrochloride	−	—	183–185°							
10	α-*Piperidinoaceto*-3: 4-*xylidide* (V)		85	71–73° (ag)	$C_{15}H_{22}ON_2$	73·0	8·9	11·4	73·2	9·0	11·4
	hydrochloride	+++	—	189–191°							
11	α-*Piperidinoaceto*-3: 5-*xylidide*		72	87–89° (ag)	$C_{15}H_{22}ON_2$	73·2	8·9	11·6	73·2	9·0	11·4
	hydrochloride	−	—	216–218°							
12	2: 4: 5-Trimethyl-α-piperidinoacetanilide		—								
	hydrochloride	+	84	246–248° (cg)	$C_{16}H_{24}ON_2$,HCl	64·6	8·5	9·2	64·7	8·5	9·4
13	2: 4: 6-*Trimethyl-α piperidinoacetanilide*		94	104–106° (ah)	$C_{16}H_{24}ON_2$	74·2	9·2	10·8	73·8	9·3	10·8
	hydrochloride	+	—	195–196° (bg)							
14	6-Amino-α-piperidinoaceto-3: 4-xylidide (IV)		—								
	dihydrochloride	−	72	272–275° (ch)	$C_{15}H_{23}ON_3$,2HCl	53·9	7·4	12·3	53·9	7·5	12·6
15	3: 4-*Dimethoxy-α-piperidinoacetanilide*		88	78–79° (ah)	$C_{18}H_{22}O_3N_2$	64·6	7·7	10·0	64·7	8·0	10·0
	hydrochloride	−	—	183–185°							
16	α-*Piperidino*-N-*diphenyl*-2-*ylacetamide*		92	134–135° (ag)	$C_{19}H_{22}ON_2$	77·6	7·4	9·4	77·5	7·2	9·5
	hydrochloride	pptd	—	169–170°							
17	α-*Piperidino*-N-*diphenyl*-4-*ylacetamide*		93	108–110° (ag)	$C_{19}H_{22}ON_2$	77·6	7·6	9·7	77·5	7·2	9·5
	hydrochloride	pptd	—								
18	p-*Chloro-α-piperidinoacetanilide*		85	86–88° (ag)	$C_{13}H_{17}ON_2Cl$	61·6	6·8	10·8	61·8	6·8	11·1
	hydrochloride	+	—	220–222°							
19	3: 4-*Dichloro-α-piperidinoacetanilide*		58	74–76° (ag)	$C_{13}H_{16}ON_2Cl_2$	54·4	5·6	9·7	54·4	5·6	9·8
	hydrochloride	++++[t]	—	196–198°							
20	5-Chloro-α-piperidinoaceto-*o*-toluidide		—								
	hydrochloride	++	87	235–237° (ch)	$C_{14}H_{19}ON_2Cl$,HCl	55·2	6·5	9·3	55·5	6·7	9·0
21	p-*Bromo-α-piperidinoacetanilide*		67	85–88° (ag)	$C_{13}H_{17}ON_2Br$	52·4	5·6	9·7	52·2	5·8	9·4
	hydrochloride	++++	—	229–231°							
22	*p*-Iodo-α-piperidinoacetanilide		—								
	hydrochloride	++++	87	218–220° (cg)	$C_{13}H_{17}ON_2I$,HCl	40·7	5·0	7·5	41·0	4·8	7·4
23	4-Chloro-2-nitro-α-piperidinoacetanilide[n]		—								
	hydrochloride	pptd	—	230–232° (bg)	$C_{13}H_{16}O_3N_3Cl$,HCl	46·5	5·3	12·7	46·7	5·1	12·6
24	2-Amino-4-chloro-α-piperidinoacetanilide										
	hydrochloride[n]	+[t]									
25	α-Piperidino-*p*-sulphamoylacetanilide		90	196–198°							
	hydrochloride	−	—	268–270° (cg)	$C_{13}H_{19}O_3N_3S$,HCl	47·0	5·8	12·4	46·8	6·0	12·7
26	α-Pyrrolidin-1′-ylaceto-3: 4-xylidide		—								
	hydrochloride	++++	78	200–203° (cg)	$C_{14}H_{20}ON_2$,HCl	62·2	7·7	10·5	62·6	7·9	10·5
27	α-*Morpholinoaceto*-3: 4-*xylidide*		52	83–85° (ah)	$C_{14}H_{20}O_2N_2$	67·7	8·3	11·5	67·7	8·1	11·3
	hydrochloride	−	—	222–224°							
28	α-Piperazin-1′-ylaceto-3: 4-xylidide		—								
	hydrochloride	++++	31	242–245° (cg)	$C_{14}H_{21}ON_3$,HCl	59·0	7·6	14·4	59·2	7·8	14·8
29	α-*Piperidinopropiono*-3: 4-*xylidide*		72	78–80° (ag)	$C_{16}H_{24}ON_2$	74·0	9·1	11·0	73·8	9·2	10·8
	hydrochloride	++++	—	178–181°							

30	α-Pyrrolidin-1′-ylpropiono-3: 4-xylidide *hydrochloride*	++++	—	78	225–227° (ch)	$C_{15}H_{22}ON_2$,HCl	63·9	8·2	9·7	63·8	8·2	9·9
31	3: 4-Dimethoxy-α-piperidinopropionanilide *hydrochloride*	—	—	78	197–199° (ch)	$C_{16}H_{24}O_3N_2$,HCl	58·2	7·5	8·7	58·4	7·7	8·5
32	*Glycyl*-3: 4-*xylidide* *hydrochloride*	—	—	87	97–99° (ag) 259–261° (cg)	$C_{10}H_{14}ON_2$ $C_{10}H_{14}ON_2$,HCl	67·3 55·9	8·0 7·0	15·3 13·0	67·4 55·9	7·9 7·0	15·7 13·1
33	α-Dimethylaminoaceto-3: 4-xylidide *hydrochloride*	—	—	48	166–168° (ch)	$C_{12}H_{18}ON_2$,HCl	59·6	7·8	11·8	59·4	7·9	11·6
34	α-Diethylaminoaceto-3: 4-xylidide hydrochloride[o]	+++	—	72	165–167° (ch)	$C_{14}H_{22}ON_2$,HCl	62·3	8·2	10·5	62·1	8·2	10·3
35	α-Dipropylaminoaceto-3: 4-xylidide *hydrochloride*	++	—	82	154–156° (dg)	$C_{16}H_{26}ON_2$,HCl	64·5	8·9	9·2	64·3	9·1	9·4
36	α-Dibutylaminoaceto-3: 4-xylidide *hydrochloride*	pptd	—	31	155–157° (fg)	$C_{18}H_{30}ON_2$,HCl	66·1	9·6	8·3	66·1	9·6	8·6
37	α-Diallylaminoaceto-3: 4-xylidide *hydrochloride*	++++	—	63	133–136° (eh)	$C_{16}H_{22}ON_2$,HCl	64·9	7·8	9·5	65·2	7·9	9·5

TABLE III

INHIBITORY ACTIVITY AND CERTAIN PHYSICAL AND CHEMICAL PROPERTIES OF 1-ARYLCARBAMOYLMETHYLPYRIDINIUM CHLORIDES AND OTHER QUATERNARY AMMONIUM SALTS

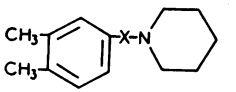


and others.

Symbols as in Table I. a–h, as in Table II; p = Mel'nikov, Sukhareva and Arkhipova (1948) record m.p. 182°; q = Mel'nikov *et al.* (1948) record m.p. 220°; r = yellow needles from dimethylformamide/ether; s = slightly toxic.

		Inhibitory Activity (0·1 mg./ml.)	Yield (%)	M.p. (Solvent, Cryst. Form)	Mol. Formula	Found (%)			Required (%)		
						C	H	N	C	H	N
38	1-*o*-Tolylcarbamoylmethylpyridinium chloride[p]	—	66	190–192° (cg)	$C_{14}H_{15}ON_2Cl$	64·3	5·8	10·4	64·0	5·8	10·7
39	1-m-*Tolylcarbamoylmethylpyridinium chloride*	—	76	218–220° (ch)	$C_{14}H_{15}ON_2Cl$	64·2	5·9	10·2	64·0	5·8	10·7
40	1-*p*-Tolylcarbamoylmethylpyridinium chloride[q]	+	67	245–247° (ch)	$C_{14}H_{15}ON_2Cl$	64·4	5·6	10·5	64·0	5·8	10·7
41	1-(2 : 3-*Xylylcarbamoylmethyl*)*pyridinium chloride*	—	43	197–199° (cg)	$C_{15}H_{17}ON_2Cl$	65·1	6·1	10·2	65·1	6·2	10·1
42	1-(2 : 4-*Xylylcarbamoylmethyl*)*pyridinium chloride*	+	52	165–167° (cg)	$C_{15}H_{17}ON_2Cl$	65·0	6·3	10·1	65·1	6·2	10·1
43	1-(2 : 5-*Xylylcarbamoylmethyl*)*pyridinium chloride*	—	64	184–186° (cg)	$C_{15}H_{17}ON_2Cl$	65·3	6·0	9·9	65·1	6·2	10·1
44	1-(2 : 6-*Xylylcarbamoylmethyl*)*pyridinium chloride*	—	50	197–199° (cg)	$C_{15}H_{17}ON_2Cl$	64·7	6·3	10·3	65·1	6·2	10·1
45	1-(3 : 4-*Xylylcarbamoylmethyl*)*pyridinium chloride* (VI)	++++	75	236–238° (cg)	$C_{15}H_{17}ON_2Cl$	64·6	6·2	10·0	65·1	6·2	10·1
46	1-(3 : 5-*Xylylcarbamoylmethyl*)*pyridinium chloride*	—	35	164–166° (ch)	$C_{15}H_{17}ON_2Cl$	65·3	6·3	9·9	65·1	6·2	10·1
47	1-(2 : 4 : 5-*Mesitylcarbamoylmethyl*)*pyridinium chloride*	—	81	190–192° (cg)	$C_{16}H_{19}ON_2Cl$	66·1	6·5	9·6	66·1	6·6	9·6
48	1-(3 : 4-*Dimethoxyphenylcarbamoylmethyl*)*pyridinium chloride*	—	60	201–203° (cg)	$C_{16}H_{17}O_3N_2Cl$	58·2	5·3	9·1	58·3	5·6	9·0
49	1-(*Diphenyl*-4-*ylcarbamoylmethyl*)*pyridinium chloride*	++++[r]	86	242–244° (cg)	$C_{19}H_{17}ON_2Cl$	69·8	5·5	8·4	70·1	5·3	8·7
50	1-m-*Chlorophenylcarbamoylmethylpyridinium chloride*	+++	76	256–258° (cg)	$C_{13}H_{12}ON_2Cl_2$	55·3	4·3	10·0	55·1	4·3	9·9
51	1-p-*Chlorophenylcarbamoylmethylpyridinium chloride*	+	62	230–232° (cg)	$C_{13}H_{12}ON_2Cl_2$	55·1	4·1	9·8	55·1	4·3	9·9
52	1-(3 : 4-*Dichlorophenylcarbamoylmethyl*)*pyridinium chloride*	++++[s]	62	282–284° (cg)	$C_{13}H_{11}ON_2Cl_3$	49·5	3·7	8·8	49·1	3·5	8·8
53	1-(5-*Chloro-o-tolylcarbamoylmethyl*)*pyridinium chloride*	+++	49	214–216° (cg)	$C_{14}H_{14}ON_2Cl_2$	56·2	4·7	9·1	56·6	4·8	9·4
54	1-p-*Bromophenylcarbamoylmethylpyridinium chloride*	+++	61	235–237° (cg)	$C_{13}H_{12}ON_2BrCl$	47·9	3·6	8·4	47·7	3·7	8·6
55	1-*p*-Iodophenylcarbamoylmethylpyridinium chloride *picrate*	++++	71	248–250° (ch) 216–218° (r)	$C_{10}H_{14}O_8N_5I$	40·4	2·9	12·1	40·1	2·8	12·3
56	4-*Methyl*-1-(3 : 4-*xylylcarbamoylmethyl*)*pyridinium chloride*	+++	68	219–222° (ch)	$C_{16}H_{19}ON_2Cl$	65·9	6·5	9·6	66·1	6·6	9·6
57	3-*Carbamoyl*-1-(3 : 4-*xylylcarbamoylmethyl*)*pyridinium chloride*	—	86	275–277° (ag)	$C_{16}H_{18}O_3N_3Cl$	60·2	5·7	13·3	60·1	5·7	13·1
58	1-*Methyl*-1-(3 : 4-*xylylcarbamoylmethyl*)*piperidinium iodide*	—	70	177–180° (ch)	$C_{16}H_{25}ON_2I$	49·3	6·4	7·0	49·5	6·5	7·2
59	1-(3 : 4-*Dimethoxyphenylcarbamoylmethyl*)-1-*methylpiperidinium iodide*	—	85	176° (ch)	$C_{16}H_{25}O_3N_2I$	45·8	6·0	6·4	45·7	6·0	6·7
60	*Triallyl*(3 : 4-*xylylcarbamoylmethyl*)*ammonium bromide*	+	59	150–152° (bg)	$C_{19}H_{27}ON_2Br$	60·4	7·3	7·4	60·2	7·2	7·4
61	1-(2-*Amino*-4-*chlorophenylcarbamoylmethyl*)-1-*methylpiperidinium iodide*	—	71	205–207° (ag)	$C_{14}H_{21}ON_3ClI$	41·1	5·2	10·0	41·0	5·2	10·2

TABLE IV
MISCELLANEOUS COMPOUNDS

Other compounds of the type  and others.

s = slightly toxic; t = toxic; u = King and Tonkin (1946); v = Walker (1940).

		X	Inhibitory Activity (0·1 mg./ml.)	Mol. Formula	Found (%)			Required (%)		
					C	H	N	C	H	N
62	*N*-(2-Piperidinoethyl)-3: 4-xylidine *dihydrochloride* (VII)	$NH.CH_2.CH_2$	++++	$C_{15}H_{24}N_2,2HCl$	59·1	8·7	9·4	59·0	8·6	9·2
63	*N*-(3-Piperidinopropyl)-3: 4-xylidine *hydrochloride*	$NH.CH_2.CH_2.CH_2$	++++	$C_{16}H_{26}N_2$,HCl	67·8	9·6	9·9	67·9	9·6	10·0
64	*N*-(2-Piperidinopropyl)-3: 4-xylidine *dihydrochloride*	$NH.CH_2.CH(CH_3)$	++++t	$C_{16}H_{26}N_2.2HCl$	59·8	8·7	8·6	60·2	8·8	8·8
65	*N*-2-(3: 4-Xylyloxy)ethylpiperidine *hydrochloride*	$O.CH_2.CH_2$	++++?s	$C_{15}H_{23}ON$,HCl	66·8	8·6	5·2	66·8	8·6	5·2
66	*N*-3-(3: 4-Xylyloxy)propylpiperidine *hydrochloride*	$O.CH_2.CH_2.CH_2$	++++?s	$C_{16}H_{25}ON$,HCl	67·4	8·9	4·7	67·7	9·2	4·9
67	1-*Piperidino*-3-(3: 4-*xylyloxy*)*propan*-2-*ol* *hydrochloride*	$O.CH_2.CH(OH).CH_2$	++++	$C_{16}H_{25}O_2N$ / $C_{16}H_{25}O_2N$,HCl	72·8 / 64·3	9·5 / 9·0	5·5 / 5·0	73·0 / 64·1	9·5 / 8·7	5·3 / 4·7
68	*N*-2-(3: 4-Xylyl)ethylpiperidine *hydrochloride*	$CH_2.CH_2$	++++?s	$C_{15}H_{23}N$,HCl	70·7	9·6	5·5	71·0	9·5	5·5
69	*N*-3-(3: 4-Xylyl)propylpiperidine *hydrochloride*	$CH_2.CH_2.CH_2$	++++s	$C_{16}H_{25}N$,HCl	72·0	9·7	5·2	71·8	9·8	5·2
70	*N*-4-(3: 4-Xylyl)butylpiperidine *hydrochloride*	$CH_2.CH_2.CH_2.CH_2$	++++t	$C_{17}H_{27}N$,HCl	72·2	9·7	4·9	72·5	10·0	5·0
71	Piperidinomethyl 3: 4-xylyl ketone *hydrochloride*	$CO.CH_2$	++	$C_{15}H_{21}ON$,HCl	67·0	8·2	5·5	67·3	8·3	5·2
72	2-Piperidinoethyl 3′: 4′-xylyl ketone *hydrochloride*	$CO.CH_2.CH_2$	++++t	$C_{16}H_{23}ON$,HCl	68·3	8·3	4·9	68·2	8·6	5·0
73	(*N*-α-Piperidinoacetylglycyl)-3: 4-xylidide *hydrochloride*	$NH.CO.CH_2.NH.CO.CH_2$	+	$C_{17}H_{25}ON_3$,HCl	59·6	7·7	12·2	59·9	8·0	12·3
74	N^1N^1-cyclo*Pentamethylene*-N^5-3: 4-*xylyldiguanide* hydrochloride	NH.C(:NH).NH.C(:NH)	++++t	$C_{15}H_{23}N_5$	65·9	8·4	25·7	65·9	8·5	25·6
75	*N*-(2-Pyrrolidin-1′-ylethyl)-3: 4-xylidine *dihydrochloride*		++++	$C_{14}H_{22}N_2.2HCl$	58·0	8·5	9·7	57·7	8·3	9·6
76	N^1N^1-*cyclo*Tetramethylene-N^5-3: 4-xylyldiguanide *hydrochloride*		++++t	$C_{14}H_{21}N_5$,HCl	56·8	7·3	23·9	56·8	7·5	23·7
77	N^1-*m*-Tolyldiguanide hydrochloride[u]		−							
78	N^1-*p*-Tolyldiguanide hydrochloride[u]		−							
79	N^1-2: 5-Xylyldiguanide hydrochloride[u]		++							
80	N^1-3: 4-Xylyldiguanide hydrochloride[u]		++++s							
81	3: 4-Xylylguanidine nitrate[u]		++++t							
82	3: 4-Dimethylbenzamidine *hydrochloride*		++++t	$C_9H_{12}N_2$,HCl	58·6	7·1	14·8	58·6	7·1	15·2
83	*N′N′*-Diethyl-N^4-(2-diethylaminoethyl)sulphanilamide hydrochloride[v]		++							
84	*N′*-2-Diethylaminoethyl-*N′N′-cyclo*pentamethylene-sulphanilamide hydrochloride[v]		++++							
85	4: 5-Dimethyl-2-(3-piperidinopropylamino)thiazole *dihydrochloride*		+	$C_{13}H_{23}N_3S,2HCl$	48·0	7·6	12·9	47·8	7·7	12·9

(N-α-*Piperidinoacetylglycyl*)-3:4-*xylidide*. — (i) The preceding base (3.6 g.) was treated with chloroacetyl chloride in glacial acetic acid in the usual manner and the resulting (N-α-*chloroacetylglycyl*)-3:4-*xylidide* crystallized from aqueous ethanol in colourless needles (2.9 g.), m.p. 190 to 192° (Found: C, 56.6; H, 5.8; N, 11.0. $C_{12}H_{15}O_2N_2Cl$ requires C, 56.3; H, 5.6; N, 11.2%). (ii) Condensation (of 1.3 g.) with piperidine (0.85 g.) in benzene afforded (*N*-α-piperidinoacetylglycyl)-3:4-xylidide (0.22 g.), m.p. 198 to 200°, and the hydrochloride separated from methanol/ethyl acetate in colourless needles, m.p. 237 to 239° (Table IV, 73).

General Method for the Preparation of 1-*Arylcarbamoylmethylpyridinium Chlorides*

The appropriate halogenoacylarylamide (1 mol.) and pyridine (1.1 mol.) were allowed to react in boiling ethanol for 4 hr. Alcohol was then removed and the products were recrystallized from methanol/ethyl acetate. The picoline (Table III, 56) and nicotinamide (Table III, 57) derivatives were similarly prepared.

The remaining quaternary ammonium salts (Nos. 58 to 61) were obtained from the appropriate tertiary amine (Table II, 11, 15, 24) and methyl iodide in hot methanol (2 hr.), or by heating the tertiary base (Table II, 37) with allyl bromide in a sealed tube at 100° for 5 hr. (Table III, 60).

N-(2-*Piperidinoethyl*)-3:4-*xylidine Dihydrochloride* (VII).—A solution of α-piperidinoaceto-3:4-xylidide (2.46 g.) in tetrahydrofuran (20 ml.) was added dropwise with stirring to crushed lithium aluminium hydride (0.76 g.) in tetrahydrofuran (30 ml.) and the mixture was boiled under reflux for 4 hr. Excess lithium aluminium hydride was destroyed by the addition of 95% ethanol, followed by water and ether, and stirring was continued for 30 min. After filtration the organic phase was separated, dried, and evaporated. A solution of the residual gum in dry acetone (20 ml.) was saturated with dry hydrogen chloride, and recrystallization of the solid, which separated, from methanol/ethyl acetate afforded colourless needles (2.30 g.) of the dihydrochloride, m.p. 176 to 178° (Table IV, 62).

In similar fashion, α-pyrrolidin-1′-ylaceto-3:4-xylidide afforded *N*-(2-pyrrolidin-1′-ylethyl)-3:4-xylidine dihydrochloride, which separated from ethanol/ether in plates (21%), m.p. 172 to 176° (Table IV, 75), and α-piperidinopropiono-3:4-xylidide, reduced for 8 hr. in di-*n*-butyl ether, gave *N*-(2-piperidinopropyl)-3:4-xylidine dihydrochloride, crystallizing from methanol/ethyl acetate in plates (64%), m.p. 180 to 182° (Table IV, 64).

N-(3-*Piperidinopropyl*)-3:4-*xylidine Hydrochloride*. —A mixture of 3:4-xylidine (12.1 g.) and 3-piperidinopropyl chloride (Marxer, 1941) (17.8 g.) in ethanol (60 ml.) was boiled under reflux for 60 hr. During this time a crystalline solid separated. The solid was dissolved by the addition of more ethanol (charcoal) and, on cooling, *N*-(3-piperidinopropyl)-3:4-xylidine hydrochloride separated in colourless needles (18 g.), m.p. 222 to 224° (Table IV, 63).

2-(3:4-*Xylyloxy*)*ethyl Chloride*.—(i) A mixture of 3:4-xylenol (12.2 g.) and ethylene oxide (5.5 g.) in ethanol (40 ml.) containing sodium ethoxide (from 0.23 g. of sodium) was heated in a sealed tube at 80° for 4 hr. The solvent was removed and the residual oil was washed in ethereal solution with 2 N-NaOH and then with water, dried, and recovered. Fractionation afforded 2-(3:4-xylyloxy)ethanol as a colourless oil (14.8 g.), b.p. 130 to 134°/1.5 mm., n_D^{25} 1.5331. (ii) The preceding product (8.3 g.) was treated in dry chloroform (50 ml.), containing pyridine (4.0 g.), with thionyl chloride (6.5 g.), and the reaction mixture was boiled under reflux for 2 hr. The solvent was removed and the product was recovered, washed, and dried in ethereal solution. Fractionation afforded 2-(3:4-*xylyloxy*)*ethyl chloride* as a colourless oil (7.25 g.), b.p. 90 to 92°/0.14 mm., n_D^{23} 1.5329 (Found: C, 64.9; H, 7.1. $C_{10}H_{13}OCl$ requires C, 65.0; H, 7.1%).

N-2-(3:4-*Xylyloxy*)*ethylpiperidine Hydrochloride*.—A mixture of 2-(3:4-xylyloxy)ethyl chloride (0.92 g.) and piperidine (0.85 g.) was heated at 140° (bath temp.) for 4 hr. and then partitioned between ether and water. Basic material was extracted by washing the ethereal solution with dilute mineral acid and recovered by basification and further extraction with ether. The crude oily base was converted to the hydrochloride in acetone in the manner outlined above, and recrystallization from methanol/ethyl acetate gave *N*-2-(3:4-xylyloxy)ethylpiperidine hydrochloride as plates (1.0 g.), m.p. 182 to 184° (Table IV, 65).

1-*Bromo*-3-(3:4-*xylyloxy*)*propane*. — A solution of 3:4-xylenol (9.2 g.) in ethanol (38 ml.) containing sodium ethoxide (from 1.73 g. of sodium) was boiled under reflux with 1:3-dibromopropane (45.5 g.) for 4 hr. The solvent was then removed and the product was recovered and washed in chloroform. Fractionation then gave 1-*bromo*-3-(3:4-*xylyloxy*)*propane* as a colourless oil (9.9 g.), b.p. 94°/0.17 mm., n_D^{19} 1.5425 (Found: C, 54.5; H, 6.3. $C_{11}H_{15}OBr$ requires C, 54.3; H, 6.2%) (see Petrow, Stephenson, and Thomas, 1956).

N-3-(3:4-*Xylyloxy*)*propylpiperidine Hydrochloride*. —A mixture of 1-bromo-3-(3:4-xylyloxy)propane (2.43 g.) and piperidine (1.7 g.) in toluene (15 ml.) was boiled under reflux for 2 hr. After cooling, the precipitated piperidine hydrochloride was collected and washed with ether, and the toluene/ether solution was evaporated. The residual oil was treated with dry hydrogen chloride in ether, and recrystallization of the resulting solid from ethanol/ether gave *N*-3-(3:4-xylyloxy)propylpiperidine hydrochloride as colourless plates (1.3 g.), m.p. 170 to 172° (Table IV, 66).

Glycide 3:4-*Xylyl Ether*.—Epichlorhydrin (13.9 g.) and 3:4-xylenol (12.3 g.) were condensed together using the general method of Petrow and Stephenson (1953) and fractionation of the product gave *glycide* 3:4-*xylyl ether* as a colourless oil (9.54 g.), b.p. 122 to 127°/1.3 mm., n_D^{20} 1.5284 (Found: C, 74.2; H, 7.9. $C_{11}H_{14}O_2$ requires C, 74.1; H, 7.9%).

1-*Piperidino*-3-(3:4-*xylyloxy*)*propan*-2-*ol Hydrochloride*.—The preceding glycide ether (1.2 g.) was heated with piperidine (0.62 g., 20% excess) under reflux for 5 hr. Fractionation of the product gave the desired base in practically quantitative yield (1.65 g.) and crystallization from aqueous alcohol gave colourless plates, m.p. 75 to 77°. The hydrochloride, prepared in acetone solution, crystallized from methanol/ethyl acetate in colourless needles, m.p. 171 to 173° (Table IV, 67).

3:4-*Xylylthioacetopiperidide*.—A mixture of 3:4-dimethylacetophenone (3.7 g.), sulphur (1.2 g.), and piperidine (3.2 g.) was heated under reflux in an oil bath for 15 hr. The resulting syrup was extracted with ether and the extract was washed with alkali, acid, and water, and evaporated. The residue was freed from volatile material by steam distillation and again recovered in ether. The residual gum (4.77 g.) solidified on standing and crystallization from *n*-propanol afforded 3:4-*xylylthioacetopiperidide* as colourless rods (2.5 g.), m.p. 83 to 85° (Found: C, 72.8; H, 8.6; N, 5.5. $C_{15}H_{21}NS$ requires C, 72.8; H, 8.6; N, 5.7%).

N-2-(3:4-*Xylyl*)*ethylpiperidine Hydrochloride*.—A mixture of 3:4-xylylthioacetopiperidide (1 g.), Raney nickel (about 3 g.) and ethanol (20 ml.) was boiled under reflux on the water-bath for 5 hr. After removal of the catalyst and solvent, the residual gum was partitioned between ether and dilute HCl. Basic material, recovered in the usual way, was converted to the hydrochloride, and recrystallization from *n*-propanol afforded *N*-2-(3:4-xylyl)ethylpiperidine hydrochloride as colourless plates (0.48 g.), m.p. 261 to 263° (Table IV, 68).

4-*Cyano*-o-*xylene*. — The diazonium salt was prepared below 5° from 3:4-xylidine (24.2 g.), conc. HCl (40 ml.) and sodium nitrite (13.8 g.) in aqueous solution (total vol. about 180 ml.), and added to a hot solution prepared from nickel chloride (47.4 g.), sodium cyanide (49 g.) and water (330 ml.). The product was isolated by steam distillation and ether extraction; the nitrile (19.5 g.) distilled at 116 to 118°/20 mm.

3:4-*Dimethylbenzaldehyde*. — 4-Cyano-*o*-xylene (26.2 g.) in ether (100 ml.) and chloroform (20 ml.) was added to a mixture of anhydrous stannous chloride (56.9 g.) and ether (250 ml.), which had been saturated with dry hydrogen chloride. Next day the aldimine stannichloride was collected and the filtrate was again saturated with dry hydrogen chloride and kept for several days, giving a further crop of solid. The complex was decomposed with hot water and the product was steam distilled and extracted with ether. Fractionation gave 3:4-dimethylbenzaldehyde as an oil (15.8 g.), b.p. 68°/0.8 mm.

β-3:4-*Xylylpropionic Acid*.—(i) A mixture of 3:4-dimethylbenzaldehyde (8.0 g.), malonic acid (6.24 g.) and pyridine (1.4 g.) was heated on the water-bath until evolution of carbon dioxide ceased. The product was purified by solution in aqueous sodium carbonate and reprecipitation; crystallization then afforded 3:4-dimethylcinnamic acid (7.6 g.), m.p. 171 to 173°, in agreement with Sugasawa and Sugimoto (1941). (ii) 3:4-Dimethylcinnamic acid (5.7 g.) was esterified with excess ethereal diazomethane (from 7.5 g. nitrosomethylurea) and the resulting methyl ester was hydrogenated in methanol (100 ml.) in the presence of palladized strontium carbonate at room temperature and atmospheric pressure. The residue obtained after removal of catalyst and solvent was treated with 25% aqueous NaOH (10 ml.) and ethanol (1 ml.) on the water-bath for 10 min. Crystallization of the recovered acid from aqueous ethanol gave β-3:4-*xylylpropionic acid* as colourless needles (4.7 g.), m.p. 82 to 84° (Found: C, 74.1; H, 8.0. $C_{11}H_{14}O_2$ requires C, 74.2; H, 7.8%).

N-3-(3:4-*Xylyl*)*propylpiperidine Hydrochloride*. — (i) The preceding acid (4 g.) was treated in dry chloroform (20 ml.) with thionyl chloride (2.5 ml.) and the mixture was boiled under reflux on the water-bath until evolution of hydrogen chloride ceased. Solvent and excess thionyl chloride were exhaustively removed *in vacuo*, and the residue was treated in dry benzene (20 ml.) with piperidine (4.2 g.). The mixture was heated for 2 hr. after the initial reaction subsided; piperidine hydrochloride was then removed and the filtrate was taken to dryness. The residue was washed in ethereal solution and then afforded an oil (5.1 g.), which slowly crystallized; the *piperidide*, sublimed at 100 to 150°/1.5 mm., had m.p. 35 to 37° (Found: C, 78.2; H, 9.4; N, 5.7. $C_{16}H_{23}ON$ requires C, 78.4; H, 9.4; N, 5.7%). (ii) The preceding piperidide (4.4 g.) was reduced in tetrahydrofuran (40 ml.) with lithium aluminium hydride (1.4 g.) in the manner described above. *N*-3-(3:4-Xylyl)propylpiperidine hydrochloride separated from ethanol/ether in colourless plates (3.3 g.), m.p. 183 to 185° (Table IV, 69).

N-4-(3:4-*Xylyl*)*butylpiperidine Hydrochloride*.—(i) Friedel-Crafts reaction between *o*-xylene (10.6 g.) and succinic anhydride (10 g.) in nitrobenzene in the normal way afforded β-3:4-dimethylbenzoylpropionic acid, crystallizing from aqueous acetic acid in prisms (14.8 g.), m.p. 129 to 131° (Found: C, 69.8; H, 6.7. $C_{12}H_{14}O_3$ requires C, 69.9; H, 6.8%). (ii) Clemmensen reduction (of 14.4 g.) in the normal way gave γ-3:4-xylylbutyric acid as a colourless oil (6.7 g.), b.p. 126°/0.04 mm. (Found: C, 75.0; H, 8.2. $C_{12}H_{16}O_2$ requires C, 75.0; H, 8.3%). Buu-Hoï, Cagniant, Hoan, and Khôi (1950) record a b.p. of 192 to 193° at 12 mm. (iii) The preceding acid (5.7 g.) was converted into the acid chloride and the piperidide in the manner described for the lower homologue, and the piperidide was analogously reduced with lithium aluminium hydride in tetrahydrofuran; *N*-4-(3:4-xylyl)butylpiperidine hydrochloride separated from benzene in colourless plates (2.75 g., 28% overall), m.p. 174 to 176° (Table IV, 70).

Piperidinomethyl 3:4-Xylyl Ketone Hydrochloride.—ω-Chloro-3:4-dimethylacetophenone (1.8 g.) and piperidine (1.7 g.) were heated in benzene (10 ml.) on the water-bath for 5 hr. Basic material was isolated by appropriate partition between aqueous and ethereal phases, and the product was converted to the hydrochloride in the usual way. Recrystallization from ethanol/ether gave piperidinomethyl 3:4-xylyl ketone hydrochloride as needles (0.82 g.), m.p. 177 to 179° (Table IV, 71).

2-Piperidinoethyl 3′:4′-Xylyl Ketone Hydrochloride. — A mixture of 3:4-*dimethylacetophenone* (1.5 g.), piperidine hydrochloride (1.22 g.), paraformaldehyde (4.5 g.), and ethanol (3 ml., containing 1 drop of conc. HCl) was heated under reflux for 1 hr. and for a further 2 hr. after the addition of more paraformaldehyde (0.3 g.). Precipitation of the product was accelerated with acetone (25 ml.), and recrystallization from methanol/ethyl acetate afforded colourless plates (1.1 g.) of the required hydrochloride, m.p. 182 to 184° (Table IV, 72).

N^1N^1-cyclo*Pentamethylene*-N^5-3:4-*xylyldiguanide.*—A mixture of 3:4-xylyldicyandiamide (Curd and Rose, 1946) (4.7 g.), piperidine (1.6 g.), hydrated copper sulphate (1.25 g.), 2-ethoxyethanol (4 ml.) and water (4.5 ml.) was heated under reflux with stirring for 2 hr., when a purple-brown complex separated. After dilution with water (60 ml.) the aqueous phase was decanted, and the residue was treated with N-HCl, when an insoluble residue (1.7 g.) of unchanged xylyldicyandiamide was rejected. The solution was treated with a concentrated solution of sodium sulphide monohydrate (3.5 g.) and charcoal, and filtered. The diguanide, precipitated on addition of excess aqueous NaOH, separated from aqueous ethanol in colourless rods (2.4 g.), m.p. 147 to 150°; the hydrochloride crystallized from methanol/ethyl acetate in colourless rosettes, m.p. 233 to 235° (Table IV, 74).

N^1N^1-cyclo*Tetramethylene*-N^5-3:4-*xylyldiguanide Hydrochloride.*—A mixture of 3:4-xylyldicyandiamide (1.9 g.), pyrrolidine (2 ml.), hydrated copper sulphate (1.25 g.), ethanol (10 ml.) and water (6 ml.) was heated under reflux with stirring for 90 min. The free diguanide, isolated as in the preceding example, was a gum and was converted in ether into the hydrochloride, which separated from methanol/ethyl acetate in colourless needles (2.35 g.), m.p. 243 to 244° (Table IV, 76).

3:4-*Dimethylbenzamidine Hydrochloride.*—A mixture of 4-cyano-*o*-xylene (12.6 g.), chloroform (25 ml.) and ethanol (10 ml.) was saturated with hydrogen chloride at 0° and kept at 2° for 3 days. Solvent and excess hydrogen chloride were removed at room temperature under reduced pressure and the residue was kept at 37° for 5 days with saturated (0°) ethanolic ammonia. Evaporation of the solvent and excess ammonia and recrystallization of the residue from methanol/ethyl acetate gave the amidine hydrochloride as colourless plates (13.6 g.), m.p. 195 to 196° (Table IV, 82).

4:5-*Dimethyl*-2-(3-*piperidinopropylamino*)*thiazole Dihydrochloride.*—A mixture of 3-piperidinopropylamine (see Whitmore, Mosher, Adams, Taylor, Chapin, Weisel, and Yanko, 1944) dihydrochloride (2.1 g.), 3-thiocyanatobutan-2-one (Gregory and Mathes, 1952) (1.3 g.) and water (2.5 ml.) was heated under reflux for 6 hr. Unchanged 3-thiocyanatobutan-2-one was removed by extracting the cooled mixture with ether. The aqueous phase was basified and extracted with ether, affording an oily base. The dihydrochloride, prepared in acetone, separated from ethanol/ether in needles (1.73 g.), m.p. 234 to 236° (Table IV, 85).

Biological Methods

Inhibition of viral synthesis was tested by observing the effect of a substance on the growth of influenza virus in small pieces of chick chorio-allantoic membrane *in vitro*. The method used was based on a modification (Tamm, Folkers, and Horsfall, 1953) of the technique of Fulton and Armitage (1951). Ten- or eleven-day chick embryos were de-embryonated, and the chorio-allantoic membranes cut into small pieces which were then stripped from the underlying shell membrane. The average wet weight of a piece of membrane was about 20 mg. The membrane pieces from a group of eggs were washed and randomized and then placed singly in $6\times\frac{5}{8}$ in. test-tubes along with 1 ml. of Earle's buffered salt solution (see Parker, 1950). In the buffer were incorporated (*a*) the substance under test, (*b*) 100 units of penicillin, (*c*) the influenza virus, strain MEL (1935) at a dilution of 10^{-3} of a stock seed kept in capillary tubes at −70°; this corresponded to 10^6 to 10^7 times the dose which infected 50% of eggs (ID50). In each test control pieces of membrane without the test substance were included and six tubes were used for each experimental group.

The tubes were stoppered and placed in a roller drum at 37° (8 rev./hr.). After 48 hr. incubation, the fluids were titrated for their haemagglutinin content and the geometric mean titres of each group were calculated. It has been found that a yield in a group of six tubes of 25% or less than that of the controls is highly significant ($P<0.01$) (Isaacs and Lindenmann, 1957). The control and experimental titres were calculated on a $\log_2$ scale, and in this report the observed degrees of inhibitory activity are expressed on a scale in which the symbols have the significance shown in Table I.

In a few control experiments it was shown that the titres of haemagglutinin in the fluids paralleled the infective titres of those fluids, both in the presence and absence of test drugs.

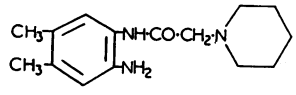
Any toxicity of drugs towards the tissues was shown by characteristic macroscopic changes in the appearance of the membranes (see Tamm, 1956). In control cultures and in the great majority of the drug experiments the membrane pieces appeared healthy at the end of the incubation period. In the presence of some drugs, however, the tissues became white and necrotic-looking, and in a few cases the suspending

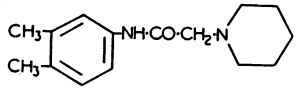
medium became stained a yellowish colour; this was taken as evidence of a severely toxic effect of the drug on the tissue.

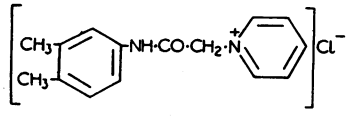
RESULTS

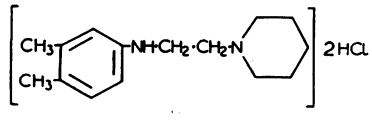
The results of tests for inhibition of virus growth under the conditions described above are recorded in Tables II to IV. It should be pointed out that it is a recognized weakness of the test that substances capable of injuring the tissue would be expected to prevent the tissue from supporting viral growth; such compounds therefore appear to be highly active although the observed inhibitory activity is exaggerated. In the absence of gross toxicity to the tissue, as was observed with the great majority of the compounds tested, the results showed clearly defined differential inhibition of virus growth. Out of 85 compounds tested, nine were obviously toxic to the tissues, three were slightly toxic, and a further three may have been slightly toxic. Four compounds could not be tested satisfactorily, as the free bases were precipitated in the buffered salt solution. Of the remaining 66 compounds, 29 showed highly significant inhibitory activity towards the virus (++, or better).

The first compound examined in the present work, 6-amino-α-piperidinoaceto-3:4-xylidide (IV) dihydrochloride (Table II, 14), completely inhibited the growth of the virus at a concentration of 1 mg./ml. and also at 0.5 mg./ml., but showed no significant inhibitory activity at 0.1 mg./ml., the concentration that was later used routinely.

(IV) 

(V) 

(VI) 

(VII) 

It was immediately noted, however, that α-piperidinoaceto-3:4-xylidide (V) hydrochloride (Table II, 10), the second compound examined, from which the 6-amino group of the compound (IV) is omitted, was considerably more active, and subsequent study was therefore directed principally towards derivatives of the much more accessible aromatic monoamines, rather than towards those of the *o*-diamines. A lengthy series of α-piperidinoacylarylamides was prepared and examined for inhibitory activity towards influenza virus in tissue culture, and these, with the biological results, are recorded in Table II. From this table a number of noteworthy features connecting structure and biological activity are readily apparent.

Of the first 25 compounds, to which the α-piperidinoacetamido group was common, the effects of variation in the aryl group are obvious. The 3:4-xylidide (V) (10) and the *m*-toluidide (4) were of comparably high activity, but omission of the 3-methyl group in compound (V) giving the *p*-toluidide (5) resulted in a fall in activity, and omission of both methyl groups in compound (V) giving the anilide (1) reduced activity still further. No activity under the conditions of the test was detected in the *o*-toluidide (3) or in the 2:6-xylidide (9), suggesting that an *o*-methyl group has an unfavourable effect upon inhibitory activity: again, the 2:5-xylidide (8) was less effective than the *m*-toluidide (4). The remaining three xylidides (6, 7, 11) were of doubtful activity. The relatively low activity of the *p*-chloro derivative (18) was markedly increased by replacing the chlorine atom by bromine (21) and by iodine (22), and replacement of both the methyl groups in the substance (V) (10) by chlorine atoms gave a compound (19) that was definitely toxic to the tissues. In eleven of the remaining compounds in Table II, the 3:4-xylidide moiety was kept constant and the side-chain was varied. It was found that the piperidino group in compound (V) could be replaced by the pyrrolidin-1-yl group (26) and the piperazin-1-yl group (28) with slightly enhanced activity, but, surprisingly, the morpholino compound (27) showed no inhibitory activity under the conditions of the test. The α-piperidinoacetyl group in compound (V) could also be replaced by the α-piperidinopropionyl group (29) and the α-pyrrolidin-1-ylacetyl group (in 26) by the α-pyrrolidin-1-ylpropionyl group (30) with unchanged high activity. The simple α-amino- (32) and α-dimethylamino-aceto-3:4-xylidide (33) were not noticeably active, but the α-dipropylamino- (35), α-diethylamino- (34), and α-diallylamino-aceto-3:4-xylidide (37) were increasingly active in that order.

The purpose of the strongly basic group in the side-chains of the compounds listed in Table II was to ensure solubility in water through the formation of soluble neutral salts. The same object

was achieved by the formation of analogous pyridinium salts which are recorded in Table III. Comparison of the relevant compounds (38 to 49, 51 to 55) with the corresponding compounds having the same aryl group in Table II shows that the same trends that were discernible in the biological activities of the piperidinoacetamido series are again discernible among the pyridinium chlorides. Of the three toluidides (38 to 40) and six xylidides (41 to 46) only the 3:4-xylidide (VI) (45) was found to be outstandingly active. An increase in inhibitory activity was again seen in passing from the *p*-chloro (51) to the *p*-bromo (54) and *p*-iodo (55) derivatives, and the 3:4-dichloro compound (52) was, as in the earlier series, slightly toxic to the tissues. Replacement of the pyridinium group in the compound (VI) by the γ-picolinium (4-methylpyridinium) group resulted in reduced activity (56), and the use of nicotinamide in place of pyridine in the quaternization reaction gave an inactive compound (57). The remaining quaternary ammonium salts (58 to 61) in Table III, obtained by quaternization of bases described in Table II with alkyl halides, were inactive or only feebly active, and it may be noted that the high inhibitory activities of the compound (V) (10) and of the diallylamino compound (37) were abolished (58) or greatly reduced (60) by this procedure.

Attention was then directed towards a study of the permissible variation of the group linking the aryl (3:4-xylyl) and basic (piperidino) groups in the compound (V), in addition to the substitution of the propionyl group for the acetyl group already noted, and the substances examined (62 to 74), together with a number of miscellaneous compounds tested, are recorded in Table IV. In those compounds in which the terminal groups were 3:4-xylyl and piperidino, high activity (and frequently also some toxicity to the tissues) was shown by all compounds in which these two groups were linked through a chain of three or four atoms: this was seemingly irrespective of the nature of the chain. One compound, (N-α-piperidinoacetylglycyl)-3:4-xylidide (73) hydrochloride, with a longer intermediate chain was doubtfully active, and one with a shorter chain (71) was only slightly active. Replacement of the piperidino by the pyrrolidin-1-yl group again gave highly active analogues (75 and 76). The simple N^1-aryldiguanides formed an interesting series, as the 3:4-xylyl representative (80) was fully active but slightly toxic, whereas the *m*-tolyl (77), *p*-tolyl (78), and 2:5-xylyl (79) derivatives were feebly or doubtfully active. 3:4-Xylylguanidine nitrate (81) and 3:4-dimethylbenzamidine (82) were both ostensibly highly active but distinctly toxic to the tissues. Isosteric replacement of the 3:4-xylyl group in the highly active *N*-(3-piperidinopropyl)-3:4-xylidine hydrochloride (63) by the 4:5-dimethylthiazolyl group (in 85) resulted in marked loss of inhibitory activity.

With a view to obtaining more insight into the mode of action of the preceding actively inhibitory compounds, *N*-(2-piperidinoethyl)-3:4-xylidine dihydrochloride (VII) (62) was selected for more detailed examination, as it possessed high intrinsic inhibitory activity towards the virus and had no obvious toxicity for the tissues.

Membranes were incubated under the standard conditions of the test described above with standard inocula of virus and compound (VII) (0.1 mg./ml.), and the virus was harvested 48 hr. later; under these conditions inhibition of the production of haemagglutinin was complete. A further set of membranes from the same large group was incubated with drug alone, and 24 hr. later the tissues were washed with fresh suspending fluid and then exposed to standard inocula of virus and incubated for a further 48 hr.; under these conditions the growth of virus was indistinguishable from that in the control tubes, indicating no permanent damage to the tissue arising from exposure to the drug and no retention of inhibitory amounts of drug by the tissue. In a further experiment the membranes were exposed to drug and virus under the standard conditions of the test. After 24 hr. the tissues were washed and re-suspended in the buffered salt solution plus penicillin; under these conditions the yield of virus 48 hr. later was actually greater than that from the control tubes, indicating effective adsorption of virus by the membranes and inhibition of growth as long as the inhibitory drug was present in the suspending fluid; after removal of the drug by washing uninhibited growth of the adsorbed virus took place.

The possibility was considered that compound (VII) might inhibit the liberation of virus from cells, rather than inhibit viral synthesis. If so, an extract prepared from infected tissue grown in the presence of compound (VII) would contain large amounts of virus. This did not, in fact, occur, but it was observed that such extracts contained complement-fixing "soluble" antigen in greater amount than would be expected from the virus yield. For instance, in one experiment the virus yield was depressed ten-fold whereas the yield of complement-fixing antigen was depressed only two-fold. It is possible, therefore, that compound (VII) exerted its effect on cytoplasmic protein synthesis to a greater extent than on synthesis of the nucleoprotein soluble antigen. Such a

differential effect has also been observed during the inhibition of the growth of influenza virus by caprochlorone in the de-embryonated egg (Liu, Malsberger, Carter, DeSanctis, Wiener, and Hampil, 1957), and a similar effect has been described for proflavine on the growth of another myxovirus, fowl plague virus (R. M. Franklin, personal communication).

Experiments were carried out to see whether the virus-inhibitory activity of the compound (VII) could be reversed by riboflavin or by vitamin B_{12}. Neither riboflavin (approx. 0.02 mg./ml.) nor vitamin B_{12} (0.001 mg./ml.), when added at the beginning of incubation, had any effect in reversing the inhibition of growth produced by the compound (VII) (0.1 mg./ml.). In the presence of 4:5-dimethyl-*o*-phenylenediamine (I) (0.001 mg./ml.), however, the virus-inhibitory activity of compound (VII) (0.1 mg./ml.) was reduced from ++++ to +. At a higher concentration of 4:5-dimethyl-*o*-phenylenediamine (0.01 mg./ml.) no antagonism of the inhibitory action of compound (VII) (0.1 mg./ml.) was observed, and at still higher concentration (0.1 mg./ml.) 4:5-dimethyl-*o*-phenylenediamine itself appeared to be markedly inhibitory (+++) towards the growth of the virus.

At the maximum tolerated dose (about 1 mg./egg) the substance (VII) had no detectable inhibitory effect on the growth of influenza virus in the chick allantoic cavity.

Most of the compounds described were examined for *in vitro* inhibitory activity towards *Streptococcus haemolyticus, Staphylococcus aureus, Escherichia coli,* and *Candida albicans,* but no outstanding activity was observed and no trends relating structure and activity were readily discernible. The majority of the compounds described in Tables II and III were examined for activity against *Trypanosoma cruzi* in the mouse, but no activity was noted.

Discussion

The biological results recorded in the present paper show that the compounds tested possessed an inhibitory action, on the reproduction of influenza virus in the chick chorio-allantoic membrane, that was markedly sensitive to changes in the chemical structures of the compounds concerned. Such inhibition of viral multiplication could be due to a number of causes operating at successive stages in virus production. Thus, (i) a direct inactivation of extracellular virus before its adsorption by the tissues, (ii) the inhibition of adsorption of the virus by the tissue, (iii) inhibition of the multiplication of the virus, or (iv) inhibition of the release of freshly synthesized virus would severally produce the gross result of an inhibition of viral growth, and experiments are described above which effectively dispose of (i), (ii), and (iv) as the causes of the observed inhibition. On the other hand a differential toxic effect on the host tissue could vary the capacity to support viral growth of a tissue in the presence of a range of drugs, but the macroscopic appearance of the tissues in the present work is against such an explanation of the finer differences observable. In all but one of the observed instances of toxicity towards the tissue the inhibition of viral growth was complete, and this particular weakness of the biological test has already been emphasized.

Our conclusion therefore is that those compounds described above, which inhibit viral reproduction without exerting any gross toxic action on the host tissue, inhibit production of viral haemagglutinin and hence production of virus particles. The effect is not due to direct inhibition of haemagglutination, to interference with the start of infection, nor to inhibition of the release of virus from cells. The virus particles produced in the presence of drug seem to be fully infective. There was significantly less inhibition of the production of influenza virus soluble antigen (which is believed to be a nucleoprotein) than of haemagglutinin in the infected tissues. The results, in fact, suggest that the drugs act specifically to inhibit the synthesis of virus haemagglutinin.

It would appear furthermore that the present observations disclose the existence of a biochemical reaction that is of greater importance for the reproduction of the virus than for the normal functioning of the host cell. Having regard to the nature of the compounds concerned and the premises which led to their examination, it was obvious that their mode of action might be related to biochemical events concerned with the functioning of riboflavin (or riboflavin-containing co-enzymes) or of vitamin B_{12}. If an inhibitor, such as compound (VII), blocked the synthesis of one or other of these metabolites the addition of the product of the blocked synthesis might be expected to reverse non-competitively the action of the inhibitor, but neither riboflavin nor vitamin B_{12} appeared to reverse the inhibition of viral growth caused by compound (VII). On the other hand, 4:5-dimethyl-*o*-phenylenediamine (I) caused a marked reduction in the inhibition produced by compound (VII) when present at 1/100th of the concentration of the inhibitor, but was itself inhibitory (or perhaps toxic) at

higher concentration. This observation therefore validates the premises upon which the work was undertaken although it is not possible to define at present the precise biochemical mechanism by which the observed inhibition is mediated. The lack of reversal of the inhibitory effect of compound (VII) in the present system by riboflavin or by vitamin B_{12} and its reversal by 4:5-dimethyl-*o*-phenylenediamine parallel the observations of Woolley and Pringle (1952) on a series of metabolite antagonists based on 4:5-dimethyl-*o*-phenylenediamine (I), modelled on quite different lines from those described in the present communication. One may also note that several of the compounds used by Woolley and Pringle (1952) and related substances have been shown (Gale and Folkes, 1957 ; Gale, 1958) to inhibit the incorporation of glycine by disrupted staphylococcal cells.

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