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Potential Chemotherapeutic Compounds—III. Derivatives of 2-Aminodiphenylamine and *N,N*-Bis(4-aminophenyl)-alkylamines

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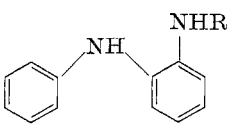
Compounds of the general structure Ar–R–Ar, in which aromatic nuclei, Ar, are separated by a single atom, R, bearing unshared electrons or a polar linkage, frequently have been found to possess tuberculostatic activity. 4,4′-Diaminodiphenyl sulphone and its derivatives are perhaps the most thoroughly studied[1-3] examples of this type but active diaryl ethers,[4-7] sulphides,[1,8-10] amines,[5,11-14] sulphoxides,[8,9] ketones[15-17] and iodonium halides[18] have been reported.

In a previous study[14] of diphenylamine derivatives, 2-aminodiphenylamine (I) showed *in vitro* tuberculostatic activity at a dilution of 1 : 100,000 in the presence of 10 per cent human serum. The compound also retarded the development of tuberculosis in guinea-pigs but proved too toxic to be considered for human treatment. Herewith are reported our efforts to convert I into less toxic derivatives suitable for use as *in vivo* tuberculostats.

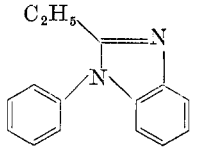
Since some toxic substances are detoxified in the body through formation of glucuronides[19,20] and several organic bases have been shown to combine with glucuronolactone,[21,22] galacturonic acid[22] or ascorbic acid[23] to give products which are less toxic than the free amines themselves, the combination of 2-aminodiphenylamine (I) with carbohydrate substances appeared to offer a promising means of detoxifying this highly tuberculostatic amine.

Attempts to condense I with D-glucose, D-galacturonic acid or L-ascorbic acid gave no well defined products but the reaction of D-glucuronolactone with 2-aminodiphenylamine in anhydrous methanol afforded a colourless crystalline product (II) of composition $C_{18}H_{18}N_2O_5$ in good yield.

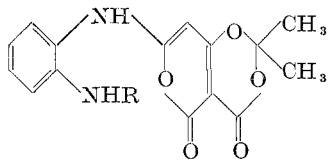
Several carboxamides (III–V) and sulphonamides (VI–IX) of 2-aminodiphenylamine also were prepared in an effort to obtain other non-toxic derivatives. In addition, 2-ethyl-1-phenylbenzimidazole (X), a cyclic analogue of 2-aminodiphenylamine (I) in which both nitrogen atoms have been incorporated into a heterocyclic ring, resulted when 2-propionamidodiphenylamine (III) was heated with hydrochloric acid. Finally, it was found that 6′-chloro-2′,4-diketo-2,2-dimethylpyrano(3′,4′:5,6)-1,3-dioxin[24] could be made to react[25] with 2-aminodiphenylamine, *o*-phenylenediamine and 2-acetamidoaniline to yield interesting new heterocyclic derivatives or analogues of I. One of these (XII) may be considered to resemble chemically[25] an acyl derivative of 2-aminodiphenylamine and so is related to the aforementioned amides (III–IX). Compounds XI and XIII are analogues of 2-aminodiphenylamine and its *N*-acetyl derivative, respectively, in which one phenyl ring has been replaced by the heterocyclic pyranodioxin nucleus. An attractive feature of this nucleus is its unsaturated lactone group, known to confer biological activity in a number of instances.[26]



I, R = H
III, R = COC_2H_5
IV, R = $CONH_2$
V, R = $CONHC_6H_5$
VI, R = $SO_2C_6H_5$
VII, R = $SO_2C_6H_4CH_3$-*p*
VIII, R = $SO_2C_6H_4NHCOCH_3$-*p*
IX, R = $SO_2C_6H_4NH_2$-*p*



X



XI, R = H
XII, R = C_6H_5
XIII, R = $COCH_3$

Representative examples (II, III, VII, VIII, X, XII, and XIII) of these derivatives showed complete tuberculostasis at dilutions of 1 : 10,000 – 1 : 20,000 in the presence of human serum, when tested against H37Rv strain of *Mycobacterium tuberculosis* var. *hominis* by the method, generally that of Doub and Youmans,[10] previously described[14] (see Table I). Compound II was only partially tuberculostatic at a dilution of 1 : 20,000.

Although II, III and VII were considerably less tuberculostatic than the parent amine (I), they were sufficiently non-toxic to be tested *in vivo*, with the hope that slow physiological hydrolysis of these compounds might liberate 2-aminodiphenylamine at such a rate as to be tuberculostatic but not toxic. However, no protective effect was demonstrated for these compounds against experimental tuberculosis in guinea-pigs or mice.

Table 1. Derivatives and analogues of 2-aminodiphenylamine.

Compound	Analysis						Highest tuberculostatic dilution	
	C, %		H, %		N, %		in medium alone	in medium containing 10% serum
	calcd.	found	calcd.	found	calcd.	found		
II	63·14	63·40	5·30	5·34	8·18	8·17	40,000*	20,000*
III	74·99	74·93	6·71	6·31	11·66	11·45	20,000	20,000
IV	68·70	68·36	5·76	5·56	18·48	18·27		
V	75·25	75·50	5·65	5·68	13·85	14·01		
VI	66·66	66·42	4·97	5·21	8·64	8·74		
VII†	67·43	67·18	5·36	5·19	8·28	8·15	20,000	20,000
VIII‡	60·99	61·10	5·61	5·71	10·16	10·40	20,000	10,000
IX	63·74	63·58	5·05	5·12				
X§					10·83	10·89	20,000	10,000
XI	59·61	59·74	4·67	4·18	9·27	8·87		
XII‖	64·36	64·11	5·62	5·34	6·01	6·24	20,000	10,000
XIII	59·28	59·30	4·68	4·90	8·14	8·30	40,000	10,000

* Partially tuberculostatic at this dilution.
† Prepared as described by F. E. King and J. W. Clark-Lewis, *J. chem. Soc.*, 3379 (1951).
‡ Contains one molecule of methanol of crystallization.
§ Analysed and tested as the hydrochloride.
‖ Contains one molecule of dioxan of crystallization.

In view of the frequency with which basic substances have been reported to show tuberculostatic activity,[3-5,27-36] it appeared of interest to prepare for testing compounds, such as XXXVI, in which the basic polar amine oxide linkage replaces the polar sulphone group of the well known tuberculostat 4,4′-diaminodiphenyl sulphone. Tuberculostatic amine oxides already have been reported[14,36] and in a series of type XXXVI there appeared to be the additional advantage that activity might be studied as a function of chain length in the alkyl substituent on the oxidized nitrogen atom.

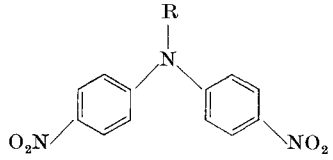
As the first step in the projected synthesis of compounds of type XXXVI, a series of *N*,*N*-bis(4-nitrophenyl)-alkylamines (XIV–XXIII) were prepared by alkylation of *N*,*N*-bis (4-nitrophenyl)-amine in the presence of aqueous potassium hydroxide. These were hydrogenated over Raney nickel to the corresponding *N*,*N*-bis(4-aminophenyl)-alkylamines, which were isolated as the free bases (XXIV–XXVI) or, more frequently, as the *N*,*N*-bis (4-acetamidophenyl)-alkylamines (XXVII–XXXIII). The tuberculostatic activities of these compounds, determined as described earlier, are shown in Tables II and III.

N,*N*-Bis(4-acetamidophenyl)-methylamine (XXVII) was oxidized to *N*,*N*-bis(4-acetamidophenyl)-methylamine-*N*-oxide (XXXIV) by means of 30 per cent hydrogen peroxide in a mixture of acetic acid and acetic anhydride at 60°. Although this substance was completely tuberculostatic at a dilution of 1 : 80,000 in the absence of serum, it was only partially tuberculostatic at 1 : 10,000 in the presence of 10 per cent serum. Hence, oxidation of the tertiary nitrogen atom in XXVII did not result in enhanced activity.

Attempts to obtain amine oxides from the amides of other *N*,*N*-bis(*p*-aminophenyl)-alkylamines were unsuccessful. Oxidation of XXXII or XXXIII yielded unidentified solid products which contained no nitrogen. Treatment of *N*,*N*-bis(4-succinimidophenyl)-*n*-hexadecylamine (XXXV) with hydrogen peroxide yielded an unidentified red solid which, however, contained nitrogen.

Hydrolysis of *N*,*N*-bis(4-acetamidophenyl)-methylamine-*N*-oxide (XXXIV) with 10 per cent hydrochloric acid, in an attempt to obtain compound XXXVI, was likewise disappointing for

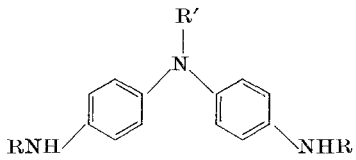
Table II. *N,N*-Bis(4-nitrophenyl)-alkylamines



Compound	Yield, %	M.p. ° C.	Analysis						Highest tuberculostatic dilution	
			C, %		H, %		N, %		in medium alone	in medium containing 10% serum
			calcd.	found	calcd.	found	calcd.	found		
XIV, R = CH_3	*								10,000†	
XV, R = C_2H_5	77	140·5	58·53	58·96	4·56	4·72	14·63	14·92	40,000	20,000
XVI, R = *n*-C_3H_7	84	132–133	59·80	59·97	5·02	4·78	13·95	14·19	20,000	10,000
XVII, R = *n*-C_4H_9	73	‡	60·94	60·85	5·44	5·70	13·33	13·51	10,000§	10,000§
XVIII, R = *n*-C_6H_{13}	50	97–98·5	62·96	63·20	6·16	6·11	12·24	12·46	20,000§	10,000†
XIX, R = *n*-C_8H_{17}	59	79–80	64·67	64·31	6·78	7·03	11·31	11·40	10,000§	10,000§
XX, R = *n*-$C_{12}H_{25}$	49	78–78·5	67·43	67·73	7·78	7·68	9·83	10·03	10,000†	10,000†
XXI, R = *n*-$C_{14}H_{29}$	59	79·5–80·5	68·55	68·44	8·18	8·00	9·22	9·27	10,000	10,000§
XXII, R = *n*-$C_{16}H_{33}$	54	80–80·5	69·55	69·33	8·55	8·24	8·69	8·78	20,000§	10,000
XXIII, R = *n*-$C_{18}H_{37}$	20	86·5–87·5	70·42	70·13	8·87	8·60	8·21	8·38	20,000§	10,000

* Prepared by the method of I. J. Pachter and M. C. Kloetzel, *J. Amer. chem. Soc.*, 74, 1321 (1952)
† Inactive at this dilution.
‡ This compound melted at 83–83·5°, then solidified and remelted at 92·5–93·5°.
§ Partially tuberculostatic at this dilution.

Table III. *N,N*-Bis(4-aminophenyl)-alkylamines and *N,N*-bis(4-acetamidophenyl)-alkylamines



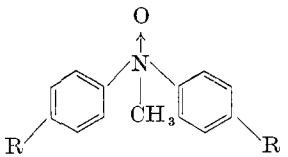
Compound	Yield, %	M.p., °C	Analysis						Highest tuberculostatic dilution	
			C, %		H, %		N, %		in medium alone	in medium containing 10% serum
			calcd.	found	calcd.	found	calcd.	found		
XXIV, R=H; R′=*n*-$C_{14}H_{29}$	92	71·5–72·5	78·93	79·23	10·45	10·41	10·62	10·81	40,000*	10,000*
XXV, R=H; R′=*n*-$C_{16}H_{33}$	77	73–73·5					9·92	9·60	20,000*	10,000*
XXVI, R=H; R′=*n*-$C_{18}H_{37}$	86	82·5–83	79·76	79·60	10·93	10·60	9·30	9·51	10,000†	10,000†
XXVII, R=CH_3CO; R′=CH_3	80	251·5–252	68·64	68·55	6·44	6·72	14·13	14·25	10,000	10,000*
XXVIII, R=CH_3CO; R′=C_2H_5	60	222·5–224	69·42	69·44	6·80	7·05	13·49	13·57	10,000*	10,000†
XXIX, R=CH_3CO; R′=*n*-C_3H_7	38	220–220·5	70·14	70·23	7·13	7·41	12·92	12·74	80,000	10,000†
XXX, R=CH_3CO; R′=*n*-C_4H_9	54	205–205·5	70·78	70·50	7·43	7·30	12·38	12·58	20,000*	10,000†
XXXI, R=CH_3CO; R′=*n*-$C_{12}H_{25}$	10	152–152·5	74·45	74·74	9·15	9·14	9·31	9·40	20,000*	10,000†
XXXII, R=CH_3CO; R′=*n*-$C_{16}H_{33}$	64	144·5–145	75·69	75·50	9·73	9·61	8·28	8·11	20,000*	10,000*
XXXIII, R=CH_3CO; R′=*n*-$C_{18}H_{37}$	57	138·5–139	76·20	76·06	9·97	9·56	7·84	8·10	10,000	10,000*

* Partially tuberculostatic at this dilution.

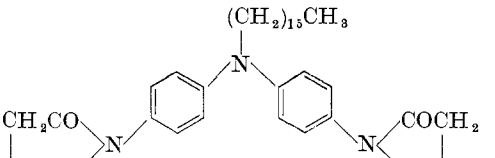
† Inactive at this dilution.

concurrent reduction took place to yield *N*,*N*-bis(4-aminophenyl)-methylamine dihydrochloride.

In a final effort to prepare XXXVI by an alternative route, *N*,*N*-bis(4-bromophenyl)-methylamine-*N*-oxide (XXXVII)[14] was heated to 140° with 28 per cent ammonia water in a pressure vessel. Again reduction occurred and the product was *N*,*N*-bis (4-bromophenyl)-methylamine.



XXXIV, R = $NHCOCH_3$
XXXVI, R = NH_2
XXXVII, R = Br



XXXV

Experimental*

Reaction of 2-Aminodiphenylamine with D-*Glucuronolactone.* A solution of 2·7 g of D-glucuronolactone and 2·76 g of 2-aminodiphenylamine (I) in 85 ml of absolute methanol was heated under reflux, with constant swirling, for 15 min, at which time crystals began to separate from the hot solution. The colourless needles which separated when the solution cooled to room temperature were filtered, washed with cold methanol, and were then suspended in 50 ml of warm methanol. The cooled suspension was again filtered, yielding 4·20 g (82 per cent) of colourless needles. The compound (II) has no distinct melting point but sinters and chars between approximately 120 and 150°. The compound decomposed when an attempt was made to recrystallize it from methanol. However, recrystallization is not necessary since the product, prepared as described, is analytically pure (see Table I).

2-Propionamidodiphenylamine (III).[37] 2-Aminodiphenylamine (18 g) was dissolved in ether (250 ml) and propionic anhydride (25 ml) was added. Upon thoroughly shaking and cooling the mixture in ice, a solid soon separated; yield, after washing with

* Microanalyses are by William J. Schenck, formerly of the University of Southern California, and Dr. Adalbert Elek, Elek Microanalytical Laboratories, Los Angeles. Melting points are uncorrected.

ether and drying, 22 g (94 per cent), m.p. 145–146°. Recrystallization from ethanol afforded colourless needles but did not raise the melting point.

o-Phenylaminophenylurea (*IV*). Potassium cyanate (0·81 g) was added to a solution of 2-aminodiphenylamine (1·84 g) in acetic acid (1·8 ml) and the mixture was heated on a steam bath for 30 min. Dilution with 20 ml of water precipitated a solid from which all colour was removed by washing with aqueous ethanol. Crystallization from aqueous ethanol afforded colourless leaflets, m.p. 153–157° (with gas evolution); yield, 1·26 g (56 per cent). Recrystallization from 95 per cent ethanol raised the m.p. to 158–159°.

N-*Phenyl*-N′-(*o-phenylaminophenyl*)-*urea* (*V*). A mixture of 2-aminodiphenylamine (1·84 g), phenyl isocyanate (1·19 g), dry petroleum ether (25 ml) and dry benzene (25 ml) was heated to reflux for 30 min. The solid which separated upon cooling was washed with aqueous ethanol to remove coloured impurities and then was crystallized from a mixture of acetone and ethanol; yield, 2·29 g (76 per cent) of colourless solid, m.p. 184–186°. Repeated crystallization raised the m.p. to 190–191°.

2-Benzenesulphonamidodiphenylamine (*VI*). To a mixture of 2-aminodiphenylamine (0·5 g), pyridine (0·25 ml) and dioxan (5 ml) was added benzenesulphonyl chloride (0·35 ml). After being boiled for several minutes the mixture was diluted with water (50 ml) and the precipitated oil was triturated with ether. Crystallization of the resulting solid from ethanol afforded 0·33 g (42 per cent) of colourless needles, m.p. 126·5–127·5°.

2-(p-Acetamidobenzenesulphonamido)-diphenylamine (*VIII*). A solution of **I** (1·84 g), *p*-acetamidobenzenesulphonyl chloride (2·34 g) and pyridine (0·85 ml) in anhydrous ether (180 ml) was allowed to stand at 30° for 19 h. The precipitated red viscous oil was washed with ether and with water and then was triturated with ethanol; yield, 2·5 g (66 per cent) of solid, m.p. 159–169°. Recrystallization from methanol afforded colourless rectangular prisms which retained one molecule of methanol of crystallization when dried in vacuum at 30° for 2 h; m.p. 98–114° (with gas evolution), resolidification and remelting at 170–171°.

When 1·0 g of this material was heated to reflux for 3 h with 5 ml of methanol and 25 ml of 5 per cent hydrochloric acid, and the

resulting purple solution was decolourized and allowed to cool, there was deposited 420 mg of colourless 2-(*p-aminobenzenesulphonamido*)-*diphenylamine* (IX), m.p. 160–162°. Crystallization from 95 per cent ethanol raised the m.p. to 161–162·5°.

2-Ethyl-1-phenylbenzimidazole (X). 2-Propionamidodiphenylamine (12 g) was heated under gentle reflux with 4 N hydrochloric acid (57 ml) for 30 min. Upon making the cooled solution basic with 10 per cent sodium hydroxide a brown oil separated. An ether extract of the mixture was dried over sodium sulphate and distilled under reduced pressure; yield, 7·5 g (68 per cent), b.p. 212° (15·5 mm). The compound was characterized as the hydrochloride.

Dry hydrogen chloride was passed into an anhydrous ether solution of the benzimidazole (4 g). The colourless hydrochloride which was precipitated was washed with ether; m.p. 195–200°. After being crystallized twice from acetone the pure hydrochloride melted at 204–206°; yield, 2·5 g (54 per cent).

6′-(2-Aminophenylamino)-2′, 4-diketo-2,2-dimethylpyrano(3′,4′ : 5,6)1,3-dioxin (*XI*). Heat was evolved when chloroform solutions of *o*-phenylenediamine (0·47 g in 10 ml) and 6′-chloro-2′, 4-diketo-2,2-dimethylpyrano(3′,4′ : 5,6)-1,3-dioxin[24] (1·0 g in 10 ml) were mixed. After being heated to boiling and cooled, the resulting solution was filtered and allowed to stand at room temperature. Compound XI separated slowly and the yield was increased by dilution of the solution with ether. Recrystallization from nitrobenzene afforded yellow microcrystals of XI which melted above 300° (dec.).

The chloropyranodioxin reacted with I in a similar manner. After being heated for 35 min the chloroform solution of the reaction product was washed several times with dilute hydrochloric acid, then with water and, after being dried, was evaporated. The residual gum gave a 60 per cent yield of crystalline XII upon trituration with ethanol. Recrystallization from dioxan afforded greenish-yellow leaflets of *2′,4-diketo-2,2-dimethyl-6′-(2-phenylaminophenylamino)-pyrano(3′,4′ : 5,6)-1,3-dioxin* (XII) containing dioxan of crystallization; m.p. 123–123·5° (with gas evolution), subsequent solidification and remelting at 240–245° (dec.).

2-Acetamidoaniline (1·3 g suspended in 10 ml of chloroform)

also reacted vigorously when added to the chloropyranodioxin (1 g in 15 ml of chloroform). *6'-(2-Acetamidophenylamino)-2', 4-diketo-2,2-dimethylpyrano(3',4' : 5,6)-1,3-dioxin* (XIII), prepared in this manner (90 per cent yield) separated from isoamyl alcohol in colourless laths, m.p. 190–190·5° (dec.).

The *2-acetamidoaniline* required for the afore-described reaction was prepared by a modification of the method of Macallum[38] in which acetic anhydride (9·5 ml) was added to a solution of *o*-phenylenediamine (10·8 g) in ether (400 ml). After being shaken vigorously and cooled, the solution eventually deposited 12·0 g (80 per cent) of the monoacetyl derivative m.p. 115–120°, suitable for use without further purification.

N,N,-*Bis(4-nitrophenyl)-amine* was prepared by modification of previously reported procedures.[39, 40] *p*-Bromonitrobenzene (280 g), *p*-nitroaniline (200 g), anhydrous potassium carbonate (100 g), cuprous iodide (4 g) and nitrobenzene (1400 ml) were heated together under reflux for 28 h. Four successive portions (1400 ml each) of nitrobenzene were added to the contents of the flask: after each addition the contents were heated to boiling and approximately 1400 ml of the hot mixture was filtered (the entire mixture was filtered after the last addition). Upon cooling, the filtrates deposited solid *N,N*-bis(4-nitrophenyl)-amine which was then subjected to steam distillation to remove *p*-nitroaniline and *p*-bromonitrobenzene. After being washed with concentrated hydrochloric acid the product was sufficiently pure for alkylation; yield, 146 g (40 per cent).

The residues from the filtration operations were extracted with further quantities of boiling nitrobenzene which, upon cooling, deposited 72 g (27 per cent) of *tris(4-nitrophenyl)-amine* in yellow hexagonal prisms, m.p. above 300°.

Anal. Calcd. for $C_{18}H_{12}N_4O_6$: N, 14·74. Found: N, 14·94.

N,N-*Bis(4-nitrophenyl)-alkylamines* (*XIV–XXIII*). In a typical instance, *N,N*-bis(4-nitrophenyl)-amine (20 g), *n*-octyl iodide (19 g), potassium hydroxide (5·1 g), water (5·2 ml) and acetone (200 ml) were heated to reflux for 2 h and then poured into water (800 ml). The precipitated solid was washed with 50 per cent aqueous ethanol (50 ml) and then with ether (50 ml). Recrystallization from ethanol afforded yellow leaflets. In Table II are listed the amines prepared in similar fashion.

N,N-*Bis(4-aminophenyl)-alkylamines* (*XXIV–XXVI*) and N,N-*bis(4-acetamidophenyl)-alkylamines* (*XXVII–XXXIII*). *N,N*-Bis(4-nitrophenyl)-alkylamines were dissolved in ethanol (15–50 ml per g) and hydrogenated over Raney nickel at a pressure of approximately 45 lb/in^2. Evaporation of the filtered solution then yielded a liquid or solid amine which, in some instances (see Table III) was purified by recrystallization from low-boiling petroleum ether. In most instances (see Table III) the crude amine was dissolved in dioxan (5 ml/g), acetic anhydride (5 ml/g) was added and the resulting solution was allowed to stand until separation of the solid diacetyl derivative appeared to be complete. Recrystallization from acetic anhydride yielded colourless plates.

N,N-*Bis(4-succinimidophenyl)-n-hexadecylamine* (*XXXV*). *N,N*-Bis(4-aminophenyl)-*n*-hexadecylamine (XXV, 500 mg) in benzene (5 ml) was added to a boiling solution of succinic anhydride (1 g) in benzene (30 ml). Upon cooling to room temperature the solution deposited a solid (probably the succinanilic acid) which was then dissolved in warm acetyl chloride (4 ml). Evaporation of the solution after 30 min yielded the crude succinanil (XXXV) which separated from absolute ethanol in colourless plates, m.p. 120–122°; yield, 400 mg (54 per cent).

Anal. Calcd. for $C_{36}H_{49}N_3O_4$: C, 73·56; H, 8·40; N, 7·15. Found: C, 73·51; H, 8·53; N, 6·93.

Oxidation of N,N-*bis(4-acetamidophenyl)-methylamine* (XXVII) was accomplished by warming a mixture of the amine (700 mg), acetic acid (30 ml), acetic anhydride (2 ml) and 30 per cent hydrogen peroxide (1·5 ml) to 60° for 90 min. Evaporation under reduced pressure left a red gum which was washed with a small amount of 5 per cent ammonia water. Crystallization (with charcoal decolourization) of the residue from water and then from 20 per cent aqueous ethanol afforded 148 mg (20 per cent) of *N,N*-bis(4-acetamidophenyl)-methylamine-*N*-oxide (XXXIV) as colourless leaflets or needles, m.p. 147·5–148°.

Anal. Calcd. for $C_{17}H_{19}N_3O_3$: C, 65·15; H, 6·11; N, 13·41. Found: C, 65·35; H, 5·85; N, 13·51.

When this *N*-oxide (66 mg) was heated to reflux for 1 h with 10 per cent hydrochloric acid (4 ml) and the resulting solution was decolourized and concentrated under reduced pressure, an infusible

salt (25 mg) separated in colourless needles. Recrystallization from water gave an analytically pure sample of *N*,*N*-bis(4-amino phenyl)-methylamine dihydrochloride.

Anal. Calcd. for $C_{13}H_{17}Cl_2N_3$: N, 14·68. Found: N, 14·75.

The salt became blue upon exposure to air and light. With silver ion, an aqueous solution of the salt gave an instantaneous precipitate of silver chloride.

Reaction of N,N-*bis*(*4-bromophenyl*)-*methylamine*-N-*oxide* (*XXXVII*) *with Ammonia.* A mixture of the oxide[14] (2·0 g) and 28 per cent ammonia water (25 ml) was heated to 140–145° in a rocking bomb for 29 h. Extraction of the reaction mixture with a mixture of ether and benzene and subsequent evaporation of the sodium hydroxide-dried extract yielded a dark residue which crystallized from 95 per cent ethanol (charcoal) in colourless needles; yield, 1·42 g (74 per cent) of *N*,*N*-bis(4-bromophenyl)-methylamine which melted at 120–121° and showed no depression of m.p. when mixed with an authentic sample of the amine.

In vivo tuberculostatic tests. 2-Propionamidodiphenylamine (III) and 2-(*p*-toluenesulphonamido)-diphenylamine (VII) were tested for their ability to prevent the development of tuberculosis in infected guinea-pigs according to the general procedure of Smith and Oechsli.[41] Beginning the same day as subcutaneous inoculation with 0·5 mg (moist weight) *M. tuberculosis* H37Rv,* each compound was administered by mouth (intrapharyngeally) in a daily dose of approximately 600 mg/kg bodyweight to 6 guinea-pigs for a period of 30 days. No protective effect could be demonstrated for either drug when compared with infected but untreated control animals, either on the basis of the tuberculin reaction or the amount of tuberculosis. The latter was measured by gross inspection, weights of lymph nodes and spleen, and by comparison of fixed spleen slices.

The condensation product (II) of 2-aminodiphenylamine with D-glucuronolactone also was tested for its ability to prevent the development of tuberculosis in animals. Thirteen mice were given approximately 3 g of the compound per kg of bodyweight per day, in their feed, beginning the day of intravenous infection with 0·1 mg (moist weight) *M. tuberculosis* H37Rv. The mean

* Provided by Dr. William Steenken, Jr., Trudeau Laboratory, Trudeau, New York.

survival period for the treated mice was 25·2 days, that for the controls 40+ days (10 of 12 were sacrificed); and there was no apparent significant difference in the amount of tuberculosis by examination or by dried lung weight.[42] Of 36 guinea-pigs infected subcutaneously with 0·1 mg (moist weight) *M. tuberculosis* H37Rv, 12 were given compound II subcutaneously in the dosage of 250 mg/kg, and 12, orally, about 600 mg/kg, daily in each case. This is about twice the possible (safe) dose of the parent compound (I) by injection and 6 times that by mouth. In the case of II, however, the injected material was poorly absorbed. The experiment was terminated at 6 weeks and assessment of the amount of tuberculosis[41] by gross estimation, and lymph node and spleen weights showed no benefit from the treatment.

Summary. New derivatives of 2-aminodiphenylamine (I), including carboxamides, sulphonamides, heterocyclic analogues and a condensation product with D-glucuronolactone, have been prepared. Several of these proved to be less toxic, but also less tuberculostatic, than the parent amine (I).

Alkylation of *N*,*N*-bis(4-nitrophenyl)-amine yielded a series of *N*,*N*-bis(4-nitrophenyl)-alkylamines (XIV–XXIII). These were reduced to *N*,*N*-bis(4-aminophenyl)-alkylamines which were subsequently converted to *N*,*N*-bis(4-acetamidophenyl)-alkylamines (XXVII–XXXIII) by acetylation. Oxidation of one of the amides (XXVII) afforded *N*,*N*-bis(4-acetamidophenyl)-methylamine-*N*-oxide (XXXIV). In the presence of human serum, the highest *in vitro* tuberculostatic dilution exhibited by any of these derivatives (XIV–XXXIV) was 1 : 20,000, when tested against H37Rv strain of *M. tuberculosis* var. *hominis*.

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