THE BACTERIOSTATIC ACTION OF BASIC DI- AND TRIPHENYLMETHANE DERIVATIVES

PART II. THE RELATIONS BETWEEN CHEMICAL STRUCTURE AND BACTERIOSTATIC EFFECTS

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THE results of bacteriostatic assays conducted with certain basic diphenylmethane derivatives, have been published in a previous paper.[1] It has been found that among different methane carbon atom derivatives of bis(*p*-dimethylaminophenyl)methane, those with a quinoid structure had a bacteriostatic activity, whereas others, without such a structure were inactive. These results were in accord with those obtained in earlier work on methane carbon atom derivatives of basic triphenylmethane dyes.[2]

The bacteriostatic potency of the active diphenylmethane derivatives (auramine and Michler's hydrol) was substantially inferior to that of triphenylmethane dyes, but the stability of their methane carbon atom substituted leuco-derivatives greatly facilitated the study of the relations between chemical structure and bacteriostatic activity.

In view of the data published by Browning and Gilmour,[3] and by Kligler,[4] related to the antibacterial activities of different triphenylmethane dyes, it was to be expected that non-methylated diaminodiphenylmethane compounds would have a weaker bacteriostatic effect than the corresponding bis-dimethyl derivatives (bis(dimethylaminophenyl)methane compounds, like Michler's hydrol and auramine).

We compared, therefore, the bacteriostatic activity of three non-methylated diaminodiphenylmethane derivatives: bis(*p*-aminophenyl)-methane, *p*-diaminobenzohydrol and *p*-diaminobenzophenone, with that of auramine and Michler's hydrol. Bis(*p*-aminophenyl)methane, the reduced non-quinoid member of the series, is the proper homologue of the triphenylmethane leucobases; *p*-diaminobenzohydrol is a potentially quinoid compound, which shows a fuchsin red colour in moderately acid solutions, corresponding to the triphenylmethane carbinol bases and to Michler's hydrol, all of them being oxidised members of their series. *p*-Diaminobenzophenone, like its homologue (Michler's ketone), is a more highly oxidised form than the carbinols, without the possibility of a tautomeric quinoid structure.

The above mentioned diphenylmethane derivatives have been prepared in our laboratory according to the methods described by Rivier and Farine[5] (bis(*p*-aminophenyl)methane and *p*-diaminobenzophenone) and by Watson and Meek[6] (*p*-diaminobenzohydrol). Melting-points: *p*-diaminodiphenylmethane, 90° C. (Rep. 87 to 94°); *p*-diaminobenzohydrol, 98° (Rep. 98°); *p*-diaminobenzophenone, 241° (Rep. 244° to 245°).

2 g. quantities of the substances to be tested were dissolved in 100 ml. of

a mixture of 4 parts of ethanol and 1 part of 0·1 hydrochloric acid. These stock solutions were then diluted with different amounts of buffered nutrient broth (*p*H 7·0). The technique of the bacteriological test was the same as in our previous experiments. The results of the assays with *Staphylococcus aureus* are shown in Table I, and with *Streptococcus fæcalis* in Table II. The results were read by comparison of the inoculated tubes with a non-inoculated control tube containing the same dilution of the test substance. The assay was repeated several times, with the same results.

TABLE I

GROWTH OF *Staphylococcus aureus* IN 24 HOURS, IN BROTH

In the presence of:	1:5000	1:10,000	1:20,000	1:40,000	1:80,000
Bis(*p*-aminophenyl)methane	+	+	+	+	+
p-Diaminobenzohydrol	±	+	+	+	+
p-Diaminobenzophenone	+	+	+	+	+
Michler's hydrol	−	−	−	−	+
Auramine	−	−	−	−	+

+ Signifies normal growth ± Signifies weak growth − Signifies no growth

TABLE II

GROWTH OF *Streptococcus fæcalis* IN NUTRIENT BROTH, IN 24 HOURS

In the presence of:	1:5000	1:10,000	1:20,000	1:40,000	1:80,000
Bis(*p*-aminophenyl)methane	+	+	+	+	+
p-Diaminobenzohydrol	±	±	+	+	+
p-Diaminobenzophenone	−	−	−	−	+
Michler's hydrol	−	−	−	−	+
Auramine	−	−	−	−	+

Summarising the results of these experiments, we may say that bis(*p*-aminophenyl)methane and *p*-diaminobenzophenone showed no bacteriostatic activity against *Staphylococcus aureus* and *Streptococcus fæcalis* in the concentrations tested. *p*-Diaminobenzohydrol caused a very slight inhibition of growth. The dimethylated substances, Michler's hydrol and auramine, showed the same degree of efficacy as in our previous experiments.[1]

The results are in accordance with our expectations, among the non-methylated bis-(*p*-aminophenyl)methane derivatives only the quinoid *p*-diaminobenzohydrol is active and its activity is considerably weaker than that of the corresponding dimethylated compound (Michler's hydrol).

We made certain observations on the effect of adding broth of *p*H 7·0 to the stock solutions of the test substances (Table III). As Table III shows, only auramine preserves its colour and solubility under these conditions. Evidently, the salt of Michler's hydrol hydrolyses at *p*H 7·0, and changes into the non-quinoid hydrol form. In other experiments we have observed, systematically, the colour and solubility of auramine and Michler's hydrol at different *p*H values. Two drops of a 1 per cent. solution of these substances, in a mixture of 70 parts of ethanol and 30 parts of 0·1N hydrochloric acid, were added to 5 ml. of buffer mixtures. The *p*H was determined potentiometrically. The colour and transparency were read after 2 hours at room temperature (Table IV).

TABLE III

COLOUR AND TRANSPARENCE OF THE NON-INOCULATED DILUTIONS OF THE TESTED SUBSTANCES IN BROTH

	Ethanolic acid stock solution (1:50)	Dilution with broth of *p*H 7		
		1:100 (Final concentration 1:5000)	1:200 (Final concentration (1:10,000)	1:400 (Final concentration 1:20,000)
Bis(*p*-aminophenyl)methane	Slightly yellow, clear	Very slightly turbid	Clear	Clear
p-Diaminobenzohydrol ..	Red, clear	Turbid, greenish colour	Slight milky opalescence; greenish	Clear
p-Diaminobenzophenone ..	Slightly yellow, clear	Slightly turbid	Clear	Clear
Michler's hydrol	Blue, clear	White, turbid	Slight milky opalescence; white	Clear
Auramine	Yellow, clear	Yellow, clear	Yellow, clear	Yellow, clear

TABLE IV

COLOUR AND TRANSPARENCE AT DIFFERENT *p*H VALUES

*p*H	Michler's hydrol	*p*H	Auramine
4·8	Blue, clear	9·4	Yellow, clear
5·2	Blue, almost clear	9·8	Light yellow, fine cryst. precipitate
5·6	Light blue, milky	10·2	Almost colourless, fine cryst. precipitate
6·0	Light blue, milky	10·6	Almost colourless, fine cryst. precipitate
6·4	White, milky	11·0	Colourless, precipitated

The differences between the behaviour of Michler's hydrol and that of auramine at different *p*H is clearly shown in Table IV. Auramine is soluble and coloured up to *p*H 9·4 and begins to lose its colour and solubility at *p*H 9·8, precipitating in the form of crystals. Michler's hydrol is soluble with a blue colour, only below *p*H 5·2; above this *p*H it gradually loses its colour and takes on a milky appearance; at *p*H 6·4 the bluish tint is entirely absent.

The different behaviour of Michler's hydrol and of auramine in these experiments, is possibly the consequence of their differing basic strength or of a lactam formation between the hydroxyl group and one of the amino groups of Michler's hydrol. Their quantitatively equal bacteriostatic effect at *p*H 7 shows that, in this case, similarities in the molecular configuration have a more marked influence on antimicrobial potency, than solubility and actually quinoid or benzenoid state. It seems, therefore, that the hypothesis forwarded by Stearn and Stearn,[7] postulating a close correlation between basic strength and bacteriostatic effects of dyes, is not fully applicable to diphenylmethane compounds. This hypothesis, originally stated in connection with observations on triphenylmethane dyes, has been successfully applied to acridine derivatives by Albert, Rubbo, Goldacre, *et al.*[8] Its validity for triphenylmethane dyes has been, however, questioned by Ingraham[9] and ourselves.[10]

Fosse[11] showed that Michler's hydrol forms a methane carbon atom semicarbazide derivative, which has a bluish colour in acetic acid. We

prepared this substance according to the description of this author, to test it in bacteriostatic experiments. We also prepared, for the same purpose, the violet coloured salt of tetrakis (dimethylaminophenyl)ethene, a compound of which the carbinol base corresponds to two molecules of Michler's hydrol joined together at the methane carbon atom (Willstätter and Goldmann[12]) and finally, the orange coloured salt of *p*-dimethylamino-triphenylmethanol (Baeyer and Villiger[13]), which is a monoamino triphenylmethane derivative.

It is a well known fact that the bacteriostatic effect of triphenylmethane dyes is inhibited in the presence of proteins. We assayed, therefore, the three above mentioned compounds, and auramine and Michler's hydrol also in the presence of 20 per cent. horse serum. The results of the bacteriostatic assay are shown in Table V, which shows that the semicarbazide

TABLE V

GROWTH OF *Staphylococcus aureus* IN BROTH

In the presence of:	Dilutions of the tested substances			
	1:40,000	1:80,000	1:160,000	1:320,000
Auramine	−	+	+	+
Auramine + 20 per cent. of serum	±	+	+	+
Michler's hydrol	−	+	+	+
Michler's hydrol + 20 per cent. of serum	±	+	+	+
Semicarbazide of Michler's hydrol	−	−	+	+
Semicarbazide of Michler's hydrol + 20 per cent. of serum	±	+	+	+
Tetrakis(dimethylaminophenyl)ethene-hydrochloride	+	+	+	+
Tetrakis(dimethylaminophenyl)ethene-hydrochloride + 20 per cent. of serum	+	+	+	+
p-Dimethylaminotriphenylmethane-hydrochloride	−	−	−	±
p-Dimethylaminotriphenylmethane-hydrochloride + 20 per cent. of serum	−	−	+	+

of Michler's hydrol is somewhat more active than Michler's hydrol and auramine. Tetrakis(dimethylaminophenyl)ethene-hydrochloride is without activity in the same concentration, whereas the monoamino triphenylmethane derivative shows the highest activity among the tested substances. Horse serum inhibits the effects of every one of the active substances. The inactivity of tetrakis(dimethylaminophenyl)ethene derivative agrees with the observations of Albert, Rubbo, Goldacre, Davey and Stone,[8] according to which, *NN'-bis*(5-acridyl)ethylenediamine, obtained by joining together two molecules of 5-methylacridine, is much less active in bacteriostatic experiments than the parent substance (dystherapeutic effect of doubling the molecular size).

Comparing the bacteriostatic activity of dimethylaminotriphenylmethanol-hydrochloride with that of Michler's hydrol and of the corresponding diaminotriphenyl- and triaminotriphenylmethane derivatives, we concluded that: (1) Whereas triaminotriphenylmethane derivatives have practically the same degree of activity as diaminotriphenylmethane derivatives (Browning and Gilmour[3]; Kliger[4]), the elimination of a second amino group from the molecule diminishes very markedly the bacteriostatic effect. (2) Eliminating one phenyl group from the triphenylmethane molecule diminishes the bacteriostatic effect in a higher

degree than the elimination of two amino groups of the same, the monoaminotriphenylmethane derivative being more effective than the diaminodiphenylmethane derivative. The maximal efficacy is reached in the aminophenylmethane series in compounds with three phenyl groups and at least two alkylated amino groups.

SUMMARY

1. *p*-Diaminobenzohydrol, a non-methylated homologue of Michler's hydrol, is less active bacteriostatically than the latter, in accordance with similar observations made on triphenylmethane dyes by Browning and Gilmour and by Kligler.

2. The potentially quinoid compounds auramine and Michler's hydrol are bacteriostatically active to an equal degree, in spite of Michler's hydrol being actually non-quinoid and auramine actually quinoid at the *p*H of the bacteriological medium.

3. The introduction of the semicarbazide radical into Michler's hydrol at the methane carbon atom augments, to a certain degree, the bacteriostatic activity.

4. The doubling of the size of Michler's hydrol to tetrakis(*p*-dimethylaminophenyl)ethanediol abolishes the bacteriostatic effect.

5. The dimethylated monoamino triphenylmethane derivative is more active than the corresponding dimethylated diaminodiphenyl derivatives, but is considerably less active than the homologous diaminotriphenylmethane and triaminotriphenylmethane compounds.

6. Serum inhibits the bacteriostatic action of all substances tested in our experiments.

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