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*The Antiseptic and Trypanocidal Action of some Benzoylamino Quinoline Anil and Styryl Compounds.**

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Biological Section.

The antiseptic and trypanocidal properties of certain amino derivatives of 2-styryl quinoline have already been dealt with.† Hitherto the substituent groups in the quinoline nucleus have been chiefly in the 6-position (occasionally the 4- or 7-position) and have consisted of primary or tertiary amino, acylamino or carboxylamino groups. In the present series the 6-position is occupied by a primary, tertiary or acetylated para amino benzoylamino group. The methods of estimating antiseptic and trypanocidal action correspond with those previously used.† The results are given in Table I. For reasons already stated (Ashley and others, p. 293), the antiseptic potency is represented by the inhibitory concentration of the substances.

Antiseptic Properties.

The anils in which the 6-amino group of the quinoline nucleus is arylated (Nos. 73, 422, 421) are all very powerfully antiseptic toward both staphylococcus and *B. coli*. In this respect they resemble the 2 *p*-dimethylamino anils in which the 6-position in the quinoline nucleus is occupied by an acylamino group (Nos. 59–69). Several of the styryl compounds also are fairly active antiseptics both for staphylococcus and *B. coli*, especially Nos. 426, 430. On comparing their effects with those of the non-benzoylated analogues

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† Browning, Cohen, Ellingworth and Gulbransen, 'Proc. Roy. Soc.,' B, vol. **100**, p. 293 (1926); *ibid.*, vol. **105**, p. 99 (1929); Browning, Cohen, Ashley and Gulbransen, *ibid.*, vol. **110**, p. 249 (1932); Browning, Cohen, Cooper and Gulbransen, *ibid.*, vol. **110**, p. 372 (1932); Ashley, Browning, Cohen and Gulbransen, p. 293.

bearing the same terminal groups in the side-chains, it appears that there is no close parallel between structure and action in the two series—*cf.* Nos. 245, 87, 427, 21 ; 426, 24 ; 437, 90. Several of the benzoylamino compounds even in very high dilutions are precipitated in protein solutions (peptone water, serum) ; this is especially so with No. 430 and, to a less extent, with Nos. 421, 437, 422 and 245.

Toxicity and Trypanocidal Action.

The substances most readily precipitated by peptone water or serum *in vitro*, also form after injection into the subcutaneous tissue coloured deposits which persist for long periods. There is a tendency for necrosis of the skin to result ; but necrosis seldom occurred with No. 430. The low toxicity of these compounds is probably due to the minute amounts which circulate in the body at a given time. In the case of No. 430, some months after the injection the material forms a mass of the colour of the original substance, with a consistency resembling that of oil paint, most of which can be easily scraped out of the connective tissue. It is readily suspended in water and appears as minute, elongated, coloured granules measuring under 1 up to several μ. No. 245 causes a greater tissue reaction and the stained mass becomes enmeshed in connective tissue from which it cannot be so readily removed. With both substances the local staining has been apparent over a year after the injection and in the case of No. 430 the development of sarcoma has been observed locally ; but with No. 245 this has not occurred in 15 animals observed for 11 to 14 months after the injection. The anil derivatives (Nos. 421, 422 and also No. 73*) have some trypanocidal action, but do not effect cure. Of the styryl compounds those with a dimethylamino group in the benzene nucleus (Nos. 426, 427) are without trypanocidal action ; the others are actively trypanocidal. No. 245 is fairly uniform in its effect, and the ratio of the dose which is tolerated to that which may produce cure is greater than 240 : 1. No. 437 does not act in so wide a range of dosage. The effect of No. 430 is highly irregular ; while a dose of 0·00025 gram may cure the infection in a mouse of 20 gram weight, on the other hand, 0·0066 gram may be entirely without effect. These substances, apparently on account of their persisting as a "depot" in the subcutaneous tissues, exert a prolonged prophylatic action. In order to give an indication of the sensitiveness to therapeutic agents of the strain of *T. brucei* used, it should be added that with similar animals treated at a corresponding stage of

* The maximum tolerated dose of No. 73 is 1 : 250.

Table I.

No.	Substance.	Antiseptic action.				Precipitation.		Maximum tolerated dose.	Trypanocidal action.	
		Staphylococcus aureus.		*B. coli.*						
		P.	S.	P.	S.	P.	S.		Dose.	Result.
422	2 (*p*-dimethylamino anil) 6 (*p*-amino benzoylamino) quinoline methochloride	>1000	200	>1000	400	40	4	>1 : 150	1 : 150–1 : 600	Slight—marked.
421	2 (*p*-dimethylamino anil) 6 (*p*-acetylamino benzoylamino) quinoline methochloride	>200	>400	>1000	100	100	40	>1 : 150	1 : 150	No action.
245	2 (*p*-acetylamino styryl) 6 (*p*-amino benzoylamino) quinoline methoacetate	100	10	1	10	20	2	>1 : 100	1 : 100–1 : 1000 1 : 2000–1 : 24000 1 : 25000 1 : 50000	Cure. (Cure). Slight—marked. No action.
427	2 (*p*-dimethylamino styryl) 6 (*p*-amino benzoylamino) quinoline methochloride	40	10	10	20	1	1	1 : 1000	1 : 1500	No action.
426	2 (*p*-dimethylamino styryl) 6 (*p*-acetylamino benzoylamino) quinoline methochloride	40	100	100	40	4	<1	1 : 1000	1 : 1200	No action.

430	2 (*p*-amino styryl) 6 (*p*-acetylamino benzoylamino) quinoline metho-acetate	100	200	20	100	1000	100	> 1 : 50	1 : 150–1 : 4000 1 : 5000–1 : 6000	No action—marked—(cure). No action—marked.
437	2 (*p*-acetylamino styryl) 6 (*p*-dimethyl-amino benzoylamino) quinoline methoacetate	< 3	20	< 3	< 3	200	20	> 1 : 100	1 : 500–1 : 2000 1 : 3000–1 : 7500	Cure. Marked.

P = medium consisting of 0·7% neutral peptone water. S = ox serum previously heated at 56° C.

Antiseptic action.—The numbers are the reciprocals ÷ 1000, of the concentrations which suffice to produce inhibition of growth in 48 hours at 37° C., so that the medium remains unclouded or shows at most very faint turbidity. In the former case subculture may yield no growth or, as in the latter case, may show very scanty growth.

Precipitation.—The numbers are the reciprocals ÷ 1000, of the lowest concentrations with which precipitation occurs in the media.

Maximum tolerated dose.—The dose shown is approximately the most concentrated solution of which a mouse weighing 20 grams will tolerate 1 c.c. injected subcutaneously without showing obvious toxic effects, *e.g.*, loss of weight.

Trypanocidal action as tested on *T. brucei* (a strain from Professor Mesnil, Institut Pasteur, Paris)—a subcutaneous injection of 1 c.c. per 20 gram mouse being given 24 hours after inoculation, when scanty parasites were present in the blood.

Lesser degrees of trypanocidal action are designated as follows:—

Slight = disappearance of parasites from the blood for several days to a week.

Marked = absence of parasites from the blood for 10 days or longer.

(Cure) indicates that with the doses shown cure was effected only in a proportion of the animals treated.

the infection with *tryparsamide* (sodium salt of N-phenylglycineamide-*p*-arsonic acid) the following results were obtained :—

Dose per 20-gram mouse.	No. of animals treated.	Result.
0·01 gram	1	1 cured
0·0066 ,,	2	1 cured 1 slight action
0·005 ,,	4	2 marked action 2 slight action
0·004 ,,	4	4 slight action
0·0033 ,,	4	3 slight action 1 no action
0·0025 ,,	9	3 slight action 6 no action

Four rabbits which had been inoculated 2 to 3 weeks previously and showed evidence of infection, as well as having parasites in their blood, received each a single subcutaneous dose of 0·02–0·025 gram per kilogram of body weight of No. 245 as a 0·7–0·8% solution in water ; no necrosis of skin resulted. All were cured.

Chemical Section.

6 p-*nitro benzoylamino quinaldine* was prepared by dissolving 10 grams of *p*-aminoquinaldine in glacial acetic acid on the water bath and adding a solution of 12 gm. of *p*-nitro benzoyl chloride in glacial acetic acid. The mixture was heated for half an hour and after standing some time to cool the precipitated hydrochloride was filtered, suspended in water and made alkaline with ammonia, filtered, washed with water and dried. 20 gm. of crude *p*-nitro benzoyl amino quinaldine were thus obtained as a white powder, which is pure enough for conversion to the amino derivative, melting point 266°–267° C. It crystallizes from nitrobenzene in pale yellow crystals. Found N 13·7%. $C_{17}H_{13}O_3N_3$ requires N 13·7%.

6 p-*amino benzoyl amino quinaldine* was obtained by reducing the 20 gm. of *p*-nitro compound in suspension in 90% acetic acid with 15 gm. of iron filings on the water bath for half an hour. The amino compound dissolved as it was formed and the precipitated iron products were removed by filtration, washed with dilute acetic acid and the diluted filtrates made alkaline with ammonia. The precipitated mixture of quinaldine compound and ferrous hydroxide was filtered, washed and extracted with boiling alcohol, filtered through a hot fluted filter, reheated to boiling and diluted with water until cloudy. On

cooling colourless needles or plates crystallized. Melting point 197°–199° C. Found N 15·2%. $C_{17}H_{15}ON_3$ requires N 15·2%. Yield 11 gm.

6 p-*amino benzoyl amino quinaldine methochloride* (Method I).—6 gm. of the base were dissolved in nitrobenzene on the water bath and 2·4 c.c. of dimethyl sulphate (redistilled below 100° C. *in vacuo*) added. After 10 minutes heating the solution was cooled and filtered, washed with nitrobenzene and benzene and dried on the water bath. The 6 gm. of methosulphate thus obtained were dissolved in the minimum quantity of hot water, filtered and precipitated with an equal volume of saturated salt solution. After cooling, filtering and washing with water, the product was dried. It can be crystallized from a large volume of water and forms small yellow needles, but is pure enough to use for condensation without crystallization.

6-p-*amino benzoyl amino quinaldine methochloride* (Method II).—11 gm. of amino quinaldine methochloride (prepared by the hydrolysis of the acetyl derivative) were dissolved in glacial acetic acid on the water bath and 8 c.c. of pyridine added. 10 gm. of *p*-nitrobenzoyl chloride in glacial acetic were then added and the mixture raised to the boiling point and refluxed for 3 hours. After allowing it to stand overnight, the crystallized product was filtered, suspended in 100 c.c. of hot water and basified with ammonia. The resulting *p*-nitro benzoyl amino quinaldine methoacetate was crystallized from 50% alcohol, forming long plates. Yield 12 gm.

This product was dissolved in 40 c.c. of 90% acetic acid and 9 gm. iron filings slowly added, the mixture being heated on the water bath between each addition and finally heated for half an hour and filtered hot. The residue was washed with a further 20 c.c. of 90% acetic acid and the solution allowed to crystallize. The crystallized portion was dissolved in hot water, made alkaline with ammonia and precipitated by salt as a bright yellow precipitate (yield 2·9 gm.). The iron oxide residues were further washed with water, the wash liquors and the acetic acid mother liquors, made alkaline with ammonia, boiled with charcoal, filtered hot and precipitated with salt (yield 7·1 gm. crude). The whole material was recrystallized from water in long yellow needles—total yield 7·0 gm. Found Cl 10·4%, $C_{18}H_{18}ON_3Cl$ requires 10·8%.

6 p-*dimethyl amino benzoyl amino quinaldine methochloride* (Method II).—6-(*p*-amino benzoyl amino) quinaldine methochloride (3·3 gm.) were suspended in a solution of 1·1 gm. of anhydrous sodium carbonate in 50 c.c. of water and 2·0 c.c. of freshly distilled dimethyl sulphate added, heated for 5 minutes on the water bath and then boiled for 10 minutes. Saturated salt solution was added and when cold the greenish yellow precipitate filtered off. The

product was suspended in absolute alcohol and water added drop by drop while boiling, till dissolved. The solution crystallized on cooling in bunches of pale greenish yellow needles (1·4 gm.). Found, Cl 10·3%, $C_{20}H_{22}ON_3Cl$ requires 10·0%.

6 p-*acetyl amino benzoyl amino quinaldine* was obtained by suspending the finely powdered amino compound in 5-6 times its weight of acetic anhydride and a little fused sodium acetate. After heating for 1 hour on the water bath, the product was filtered, washed with water and dilute ammonia and dried. The white powder was used directly for the preparation of the methochloride. It crystallized from alcohol in stout needles. Melting point above 300° C. Found N 13·3%, $C_{19}H_{17}O_2N_3$ requires N 13·2%.

6 p-*acetyl amino benzoyl amino quinaldine methochloride.*—4 gm. of the base were dissolved in the minimum quantity of nitrobenzene at 150° C. (about 250 c.c.) and 1·6 c.c. of freshly distilled dimethyl sulphate added. The temperture was maintained at 150° C. for a quarter of an hour and the mixture then allowed to cool slowly. The product was then treated as for the *p*-amino methochloride preparation.

6 p-*dimethyl amino benzoyl amino quinaldine methiodide* (Method I).—8·4 gm. of the amino benzoylamino quinaldine were suspended in a solution of 3·6 gm. anhydrous sodium carbonate in 50 c.c. of water. 10 c.c. of dimethyl sulphate were added and the mixture warmed gently. A vigorous reaction started and a clear solution resulted. The solution was boiled for 10 minutes and then evaporated to a small bulk. A solution of 15 gm. of potassium iodide was next added and the yellow precipitate of methiodide filtered. It was crystallized from about 1 litre of water. It forms yellow microscopic crystals. Found I 27·9%, $C_{20}H_{22}ON_3I$ requires I 28·4%.

Condensations with p-*dimethyl amino,* p-*amino and* p-*acetyl amino benzaldehydes.*

The following conditions had to be adhered to in all these condensations since the adoption of the usual methods (ethyl alcohol and water to dissolve the quinaldinium salt) gave dark coloured, black or even tarry products.

3 gm. of the dry powdered methochloride were suspended in about 100 c.c. of methyl alcohol, a slight excess of aldehyde and 1 c.c. of piperidine added. The mixture was then gently refluxed for 6 hours and filtered hot. The resulting chloride or iodide was too insoluble in some cases and was converted to the methoacetate by refluxing in methyl alcohol with a slight excess of

silver acetate for 1 hour. The methoacetate was then dissolved out with hot methyl alcohol and distilled off at a low temperature till crystallization took place.

The following products were prepared :—

2 (p-*acetylamino styryl*) 6 (p-*amino benzoyl amino*) *quinoline methoacetate* (245)—orange red crystals, giving an orange solution in water. Found N 11·2%, $C_{29}H_{28}O_4H_4$ requires 11·3%.

2 (p-*dimethyl amino styryl*) 6 (p-*amino benzoyl amino quinoline methochloride* (427)—dark green needles, giving a violet solution in water. Found Cl 7·4%, $C_{27}H_{27}ON_4Cl$ requires Cl 7·7%.

2 (p-*dimethyl amino styryl*) 6 (p-*acetyl aminobenzoyl amino*) *quinoline methochloride* (426)—dark brick-red crystals with slight green reflex, giving violet red solution in water. Found Cl 6·6%, $C_{29}H_{29}O_2N_4Cl$ requires 7·1%.

2 (p-*amino styryl*) 6 (p-*acetyl amino benzoyl amino*) *quinoline methoacetate* (430)—brick-red crystals with slight green reflex, giving red solution in water. Found N 10·9%, $C_{29}H_{28}O_4N_4$ requires 11·3%.

2 (p-*acetyl amino styryl*) 6 (p-*dimethyl amino benzoyl amino*) *quinoline methochloride*—orange-yellow crystals, giving a yellow solution in water. The methochloride prepared from the dimethylamino benzoyl amino quinaldine methochloride made by method II was purer than that from a product prepared by method I. Found Cl 7·1%, $C_{29}H_{29}O_2N_4Cl$ requires 7·1%.

The corresponding *methoacetate* (437) was obtained by refluxing the methochloride in alcoholic solution with silver acetate, filtering and evaporating till crystallization took place. Orange crystals, giving a yellow-orange solution in water.

Condensation with p-*nitroso-dimethylaniline.*

Anil condensations took place satisfactorily with the usual conditions (ethyl alcohol and water to dissolve the quinaldinium salt).

2 (p-*dimethylamino anil*) 6 (p-*acetylamino benzoylamino*) *quinoline methochloride* (421)—long needles with copper reflex, changing to a green reflex on drying on water bath, and dissolving in water to a deep blue solution. Found Cl 7·2%, $C_{28}H_{28}O_2N_5Cl$ requires 7·1%.

2 (p-*dimethylamino anil*) 6 (p-*amino benzoylamino*) *quinoline methochloride* (422)—crystals with a green-gold reflex, giving a blue solution in water. The yield was poor.