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*The Antiseptic and Trypanocidal Action of certain Styryl and Anil Benzthiazole Derivatives.**

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In view of the powerful antiseptic action of certain anil quinoline compounds (Browning, Cohen, Ellingworth and Gulbransen, 1926) and the trypanocidal action of these and especially of some styryl quinoline derivatives (Browning, Cohen, Ellingworth and Gulbransen, 1929), it was thought desirable to examine the action of similar compounds containing other heterocyclic nuclei. The present communication is concerned with a number of derivatives of benzthiazole, in which the pyridine ring of the quinoline group is replaced by a five-membered ring containing both nitrogen and sulphur.

Antiseptic Action.

Antiseptic power was estimated as in previous communications (Browning, Cohen, Ellingworth and Gulbransen, 1926, 1928). Throughout the range of the anil benzthiazole compounds examined the antiseptic activity is considerably lower than that of the corresponding quinoline compounds. The styryl compounds tend also to be weak in their antiseptic action and in this respect resemble the styryl quinoline series, but in general they are not inferior to the latter in potency. Frequently in the present series of compounds the irregularities in action, previously commented upon,† were observed in marked degree.

Comparison of Styryls and Anils.—In the quinoline series, the anils were distinctly more potent than the corresponding styryls, especially against *B. coli*. The styryl and anil benzthiazoles, on the other hand, do not exhibit any marked contrast. In several cases (*cf.* 349, 336; 253, 252; 363, 362;

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† Browning, Cohen, Gaunt and Gulbransen, 'Proc. Roy. Soc.,' B, vol. 93, p. 329 (1922).

365, 364; 330, 329) the styryl compound is slightly more potent against staphylococcus in peptone water than is the corresponding anil.

Effect of Serum.—The action in the presence of serum appears to differ according to the organism used. Whereas in most cases the action on staphylococcus is greatly reduced by the presence of serum, there is in general a tendency towards increased potency against *B. coli* in serum when compared with peptone water.

Substitution in the Benzene Nucleus.—In the styryl group, the relative effects of a primary, secondary, tertiary or acetylamino group are such as would be expected in view of the effect of similar changes in the styryl quinolines. Thus, the amino styryl (254) is less active than the dimethylamino compound 253, and substance 332 (amino) is slightly less potent than either 349 (methylamino) or 330 (dimethylamino). In the anil series there is little difference in activity between the primary, secondary and tertiary compounds (*cf.* 335, 336 and 329). The acetylamino styryl compounds 368 and 333, as in the case of the corresponding quinoline derivatives, have little or no antiseptic action; but No. 377, like its quinoline analogue No. 90, has more pronounced action on staphylococcus. The acetylamino anil derived from nitroso tetrahydroquinoline (338) has also a powerful action on staphylococcus in peptone water.

Substitution in the Benzthiazole Nucleus.—In the previous communications regarding the quinoline compounds, it was observed that in general an increase in the mass of the quinoline nucleus tended to increase the antiseptic power. The same tendency was shown to some extent in the benzthiazoles. Thus, the α- and β-naphthathiazole derivatives 363, 365 (styryl) and 362, 364 (anil) are rather more active than the corresponding benzthiazole compounds 253 and 252.

In the anil compounds the introduction of an acetylamino group, however, did not increase the antiseptic action (252, 329); whereas in the corresponding substances of the anil quinoline series this was a marked feature. The chloracetyl anil derivatives (340, 341) appeared to be practically equal to the acetyl compound 329.

It should be noted that no attempt has been made to prepare a complete series of derivatives for comparison of antiseptic action, since it became evident at an early stage of the work that the group as a whole was greatly inferior to the anil quinoline compounds in this respect. Later, compounds were prepared solely with a view to investigating trypanocidal action.

Trypanocidal Action.

The benzthiazole derivatives, both anil and styryl, exhibited trypanocidal action when appropriate groups were present in the respective nuclei. This effect was tested for as before, in mice infected with *T. brucei*, treatment being administered 24 hours after inoculation, when scanty parasites were present in the blood. The necessary groups proved to be identical with those which produced trypanocidal action in the quinoline series. Thus the maximum effect was produced when one nucleus contained a basic group, and the other an acetylamino group. As in the quinoline series, the styryls were much more powerful and definite in action than the anils.

Cures were obtained with the following compounds which contain substituent groups as shown :—

Styryl No.	Group in benzene nucleus.	Group in benzthiazole nucleus.
332	NH_2	$NH.COCH_3$
349	$NH.CH_3$	$NH.COCH_3$
330	$N(CH_3)_2$	$NH.COCH_3$
368	$NH.COCH_3$	NH_2
333	$NH.COCH_3$	$NH.COCH_3$
377	$NH.COCH_3$	$N(CH_3)_2$

In the anil benzthiazole group, some action was observed with all the compounds except No. 252, but only with No. 336 was cure obtained. A striking feature of No. 329 was the tendency for therapeutic action to occur on a wide range of dosage (1/150 to 1/4000), although even the largest dose failed to produce cure.

In the quinoline series, the anil possessing the strongest trypanocidal action was that containing a dimethylamino group in the benzene nucleus, and a chloracetylamino group in the quinoline nucleus. No cure was observed with the corresponding benzthiazole derivatives 340 and 341.

Anil Benzthiazoles.

No.	Substance.	Antiseptic action.												Precipitation.		Trypanocidal action.	
		Staphylococcus aureus.						*B. coli.*									
		Peptone water.			Serum.			Peptone water.			Serum.			In peptone water.	In serum.	Dose.	Result.
		+	±	−	+	±	−	+	±	−	+	±	−				
335	2 (*p*-amino anil) acetylamino benzthiazole . Me_2SO_4	40	20	4	10	4	2	10	4	2	40	20	10	10	2	1/2000	Slight
336	2 (*p*-methylamino anil) acetylamino benzthiazole . Me_2SO_4	200	40	20	40	20	10	2	—	1	200	100	40	10	2	1/2000 1/4000	(Cure) Trace
252	2 (*p*-dimethylamino anil) benzthiazole . MeCl	200	100	40	20	—	10	1	—	?	2	—	1	10	4	1/2500	0
362	2 (*p*-dimethylamino anil) *α*-naphthathiazole . Me_2SO_4	400	200	100	100	40	20	10	2	1	40	20	4	2	—	1/600	Trace
364	2 (*p*-dimethylamino anil) *β*-naphthathiazole . Me_2SO_4	1000	400	200	400	200	20	100	40	4	100	40	10	10	—	1/200	Trace
329	2 (*p*-dimethylamino anil) acetylamino benzthiazole. Me_2SO_4	100	40	1	40	20	?	1	—	?	20	10	?	10	2	1/150 1/500 1/4000	Marked Slight
341	2 (*p*-dimethylamino anil) chloracetylamino benzthiazole . MeCl	40	20	1	4	—	2	1	—	?	10	—	4	10	—	1/200	Slight

340	2 (*p*-dimethylamino anil) chloracetylamino benzthiazole . Me_2SO_4	40	20	1	10	—	4	2	1	?	10	—	4	20	—	1/200	Slight
338	Condensation product of nitroso tetrahydro quinoline and acetylamino benzthiazole . Me_2SO_4	400	—	200	100	40	20	40	—	20	40	20	10	4	—	1/1500	Trace

Antiseptic action.—In this and the following table the numbers are the reciprocals ÷ 1000, of the concentrations which produce the effects shown in 48 hours at 37° C.

\+ = free growth of the organisms with the development of marked turbidity of the medium.

± = inhibition of growth, the medium being only faintly turbid or remaining unclouded, but in the latter case sub-cultures yielding growth.

— = complete sterility as tested by the absence of growth in sub-cultures.

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

Trypanocidal action.—The doses shown are reckoned per 20 gram body-weight of the mouse. The highest dose shown for each substance is one which approximates to the largest amount borne by uninfected animals without producing obvious toxic effects, *e.g.*, loss of weight.

The terms used to designate the lesser degrees of trypanocidal action are as follows :—

Trace = prolongation of life for several days beyond that of the untreated controls.

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.

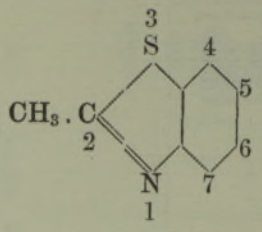
Styryl Benzthiazoles.

No.	Substance.	Antiseptic action.												Precipitation.		Trypanocidal action.	
		Staphylococcus aureus.						*B. coli.*									
		Peptone water.			Serum.			Peptone water.			Serum.			In peptone water.	In Serum.	Dose.	Result.
		+	±	−	+	±	−	+	±	−	+	±	−				
254	2 (*p*-amino styryl) benzthiazole . MeCl....	200	100	20	10	—	4	20	10	4	4	—	2	—	—	1/10000	0
332	2 (*p*-amino styryl) acetylamino benzthiazole . Me_2SO_4	40	20	10	40	20	10	1	—	?	10	4	?	4	2	1/1500 1/5000 1/10000	(Cure) Slight
349	2 (*p*-methylamino styryl) acetylamino benzthiazole . Me_2SO_4	200	—	100	20	—	10	10	—	4	10	—	4	—	—	1/1500 1/2500 1/5000 1/7500 1/10000	Cure (Cure) Slight
253	2 (*p*-dimethylamino styryl) benzthiazole . MeCl	400	200	100	40	20	10	100	—	40	40	—	20	4	—	1/10000	0
363	2 (*p*-dimethylamino styryl) α-naphthathiazole . Me_2SO_4	?	400	200	200	100	40	100	40	2	20	10	4	20	—	1/2000	Trace
365	2 (*p*-dimethylamino styryl) β-naphthathiazole . Me_2SO_4	2000	—	1000	200	100	40	20	10	4	40	20	10	10	—	1/2500	Trace

330	2 (*p*-dimethylamino styryl) acetylamino benzthiazole . Me_2SO_4	200	100	40	40	—	20	1	—	?	20	10	4	1	1/1500 1/5000 1/7500 (Cure) Trace
368	2 (*p*-acetylamino styryl) amino benzthiazole . MeCl	2	1	?	4	2	1	1	—	?	10	4	1	—	1/1000 1/5000 1/7500 1/10000 Cure Marked
333	2 (*p*-acetylamino styryl) acetylamino benzthiazole . Me_2SO_4	1	—	?	4	—	2	1	—	?	1	—	?	—	1/600 1/1500 1/5000 Cure Trace
377	2 (*p*-acetylamino styryl) dimethylamino benzthiazole . MeCl	400	200	100	20	—	10	1	—	?	10	4	1	—	1/1000 1/2000 1/2500 1/5000 1/10000 Cure Marked

Chemical Section.

The following system of numbering has been used :—



2-*Methyl benzthiazole* was made according to the method of Jacobson (' Ber., vol. 19, p. 1072 (1886)). It was found difficult to obtain a uniform yield in different preparations, much apparently depending upon the rate of melting of the mixtures of acetanilide and phosphorus pentasulphide. It was essential that these reagents should be thoroughly well ground up together, and after solution of the product in alcohol and subsequent dilution, it was necessary to regulate fairly accurately the addition of alkali in order to allow precipitation of the crystalline by-product, and to obtain the main product in solid form.

The thio-acetanilide, purified as described, was almost colourless and melted sharply at 76°. The maximum yield obtained was 51 per cent. of the weight of acetanilide taken, compared with 25 to 30 per cent. claimed by Jacobson. This is equivalent to 45·6 per cent. of the theoretical.

The oxidation of the thioacetanilide was carried out as described by Jacobson, the same yield being obtained.

2-*Methyl benzthiazole methochloride.*—Equivalent quantities of the base and dimethyl sulphate were mixed and warmed gently on the water-bath. Vigorous combination soon occurred, and a dark viscid mass was formed, which soon crystallised. It was converted to the methochloride by boiling the aqueous solution with hydrochloric acid and barium chloride. The filtered solution was evaporated to dryness and the product extracted with absolute alcohol, from which, on concentration, the required methochloride separated in almost colourless crystals.

Amino 2-*methyl benzthiazole.*—The nitro derivatives was first prepared by the direct nitration of 2-methyl benzthiazole. 2-methyl benzthiazole (14·9 gm.) was dissolved with cooling in 45 c.c. concentrated sulphuric acid. A mixture of 14·9 c.c. concentrated nitric acid (sp. gr. 1·42) and an equal volume of concentrated sulphuric acid was added gradually, the mixture being cooled in ice water and the temperature kept below 10°. After standing for 20

minutes at room temperature the mixture was poured into a large volume of ice water. The sulphate of the nitro compound, which separated, was filtered off, mixed into a cream with water, and made alkaline with ammonia. The product was recrystallised from alcohol, and formed colourless needles, melting at 165–166°. The yield was 12 gm. equivalent to 61·8 per cent. of the theory.

0·1510 gm. gave 19·4 c.c. N at 18° and 747 mm. N = 14·69 per cent.
$C_8H_6O_2N_2S$ requires N = 14·43.

The amino derivative was obtained by reduction of the nitro compound in the ordinary way with stannous chloride and hydrochloric acid. It was recrystallised from water, and formed long colourless needles melting at 124–125°. The yield on reduction was 76 per cent. of the theory.

0·3197 gm. gave 48·4 c.c. N at 19° and 751 mm. N = 17·24 per cent.
$C_8H_8N_2S$ requires N = 17·08 per cent.

Acetyl amino 2-methyl benzthiazole was obtained by heating the amino compound on the water bath with half its weight of fused sodium acetate and excess of acetic anhydride. The diluted mixture was made alkaline with ammonia, and the product filtered off and recrystallised from boiling water, with the addition of a little charcoal. It formed colourless prisms melting at 149–150°.

0·3041 gm. gave 35·4 c.c. N at 11° and 750 mm. N = 13·71 per cent.
$C_{10}H_{10}ON_2S$ requires N = 13·59 per cent.

Acetylamino 2-methyl benzthiazole methosulphate was prepared by heating the base in nitrobenzene solution with a slight excess of dimethyl sulphate at 100°. The resulting product was filtered, and thoroughly washed with ether. It was then used directly for condensation. On crystallisation from alcohol it yielded colourless crystals.

0·3236 gm. gave 23·5 c.c. N at 12·5° and 746 mm. N = 8·45 per cent.
$C_{12}H_{16}O_5N_2S_2$ requires N = 8·43 per cent.

Acetylamino 2-methyl benzthiazole methochloride, obtained as in the case of the corresponding quinaldine derivative, was salted out from the concentrated aqueous solution of the methosulphate with sodium chloride.

Amino 2-methyl benzthiazole methochloride.—The acetylamino methochloride, prepared as above, was boiled for 1 hour with concentrated hydrochloric acid. The solution was cooled, diluted, neutralised with ammonia,

and saturated with sodium chloride. The amino methochloride crystallised on standing, and was filtered, pressed, and dried. It was used for condensation without further purification.

Dimethyl amino 2-methyl benzthiazole methiodide.—An attempt was first made to prepare this compound from amino 2-methyl benzthiazole in one operation, as described in the former paper for 6-dimethyl amino quinaldine. The amino 2-methyl benzthiazole (1 mol.) was boiled with an aqueous solution of sodium carbonate (1 mol.) and methyl *p*-toluene sulphonate (3 mols.). Instead of a clear solution, however, an oil, partly soluble in the hot solution, was formed. After cooling, this oil was extracted with chloroform, the extract dried, and the solvent removed. The residual oil solidified in a freezing mixture, but melted again on warming to room temperature. All attempts to crystallise it were unsuccessful. The product did not contain iodine, and it appeared that the amino group had been methylated (since CO_2 was evolved vigorously during the preparation) without the formation of the quaternary group.

The product was finally obtained by heating the oil described above with excess of methyl iodide in toluene or alcoholic solution in a sealed tube for 3 to 5 hours at 100 to 110°. The resulting cystalline deposit was filtered off and recrystallised from aqueous alcohol containing some sulphur dioxide (to remove any periodide).* Small yellow crystals were obtained, which on drying at 100° *in vacuo*, became colourless owing to loss of water of crystallisation.

0·1614 gm. gave 0·1144 gm. AgI, I = 38·32 per cent.
$C_{11}H_{15}N_2SI$ requires I = 38·00 per cent.

Anil benzthiazole Derivatives.—These were all prepared by condensation of the benzthiazole quaternary derivative in alcoholic or aqueous alcoholic solution with the appropriate nitroso compound. They are very similar in appearance to the corresponding quinoline compounds, and crystallised from the alcoholic solution in prisms showing a blue or bluish green reflex. They form blue or blue-violet solutions in alcohol, the aqueous solutions being of a redder shade. Similar compounds have been previously described by Smith (1923), and Hamer (1929).

No piperidine was necessary to effect these condensations.

Styryl benzthiazole Derivatives.—These compounds were obtained in the

* In the absence of sulphur dioxide the iodine content was too high.

same way as the anils, the nitroso compound being replaced by the appropriate aldehyde. Piperidine was in all cases used to accelerate the reaction.

The products, as in the case of the quinoline compounds, gave redder solutions, both in alcohol and water, than the corresponding anils.

2 (*p-Acetylamino styryl*) *amino benzthiazole methochloride* separated from the alcoholic solution in scarlet needles (or flat prisms) depending upon the temperature of separation).

0·2719 gm. (dried at 100° *in vacuo*) gave 26·9 c.c. N at 16·5° and 774 mm. N = 11·76 per cent.

$C_{18}H_{18}ON_3SCl$ requires N = 11·68 per cent.

2 (*p-Acetylamino styryl*) *acetylamino benzthiazole methosulphate* crystallised from the boiling alcoholic solution, in which it was prepared, in orange plates or flat prisms. It formed an orange solution in water.

2 (*p-Acetylamino styryl*) *dimethylamino benzthiazole methochloride*.—Dimethylamino benzthiazole was obtained from the amino compound by the method described above. The methiodide (1·1 gm.) was dissolved in alcohol and heated, if necessary, to remove sulphur dioxide and *p*-acetylamino benzaldehyde (0·6 gm.) added with three drops of piperidine. After boiling for 3½ hours the product was filtered hot, dried and analysed.

0·2423 gm. gave 18·7 c.c. N at 18° and 751 mm. N = 8·84 per cent.

$C_{20}H_{22}ON_3SI$ requires N = 8·77 per cent.

It was converted into the methochloride by boiling the methyl alcohol solution with silver chloride and evaporating to dryness after filtering off the silver iodide.

We are indebted to Dr. K. E. Cooper for the preparation of considerable quantities of methylbenzthiazole, its nitro and amino derivatives.

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