Chinonen en chinonderivaten en hun invloed op micro-organismen

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SUMMARY.

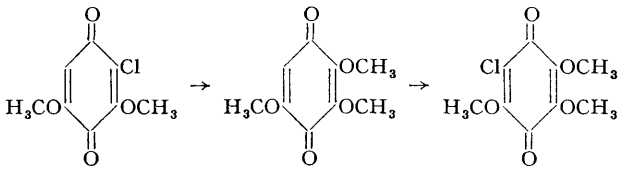
The second chapter of this thesis contains a survey of the most important antibiotics, which have been isolated up to the present. It will be seen that several of them are derivatives of quinones; a disadvantage is often their toxicity.

We therefore tried to prepare other quinones in the hope of obtaining active compounds of lower toxicity.

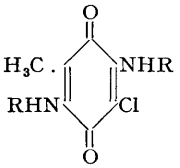
In the thesis are described a number of derivatives of p-benzoquinone and p-toluquinone, which possess other groupings than the natural antibiotics of the quinone type viz. fumigatin (3-hydroxy-4-methoxy-2,5-toluquinone) and spinulosin (3,6-dihydroxy-4-methoxy-2,5-toluquinone) and which differ from the quinones prepared and tested by Oxford and his collaborators.

The preparation of a number of hydroquinones and of hydroquinone esters is also described. The compounds prepared are listed in the left of Table I *).

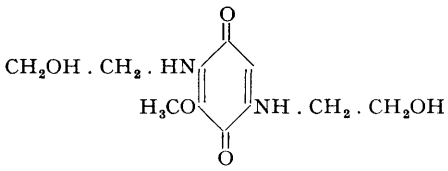
The unknown 3-chloro-2,5,6-trimethoxybenzoquinone-1,4 was prepared as follows:



Diaminoquinones of this formula have been synthesized by the reaction of trichlorotoluquinone-2,5 with amines.



2-Methoxy-3,6-bis-oxyethylaminobenzoquinone-1,4 has resulted from the reaction between 2,6-dimethoxybenzoquinone-1,4 and aminoethanol.



*) The Tables will be found at the end of this thesis.

By reducing the quinones with $SnCl_2$ and diluted hydrochloric acid the corresponding hydroquinones were prepared.

The di-esters (diacetates and dibutyrates) of the hydroquinones have been obtained by reducing the quinones with zinc in a mixture of the organic acid and its anhydride („reductive acylation").

Diphosphates and bis-p-aminobenzoates were prepared from hydroquinones and phosphorus oxychloride, or p-aminobenzoyl-chloride hydrochloride respectively.

2,6-Dimethoxyhydroquinone octa-acetyl-bis-β-glucoside has been synthesized from the hydroquinone and β-penta-acetyl glucose in toluene using p-toluene sulphonic acid as a catalyst.

However, on saponifying the octa-acetate in alcaline alcohol the compound decomposed.

The biological activity of all these compounds, so far as they are soluble in water, has been tested *in vitro* against a yeast, various moulds and Gram-positive and Gram-negative micro-organisms.

The toxicity has been determined in white mice, by oral administration of the quinones, hydroquinones and di-esters and by intraperitoneal injection of the di-esters. The chemotherapeutic activity has been investigated in white mice injected with a lethal dose of pneumococci.

In Table I, figures are given for the inhibiting effects of these compounds on the growth of the yeast *Saccharomyces cerevisiae* and of the moulds *Aspergillus niger*, *Mucor racemosus*, *Penicillium meleagrinum* and *Penicillium notatum*.

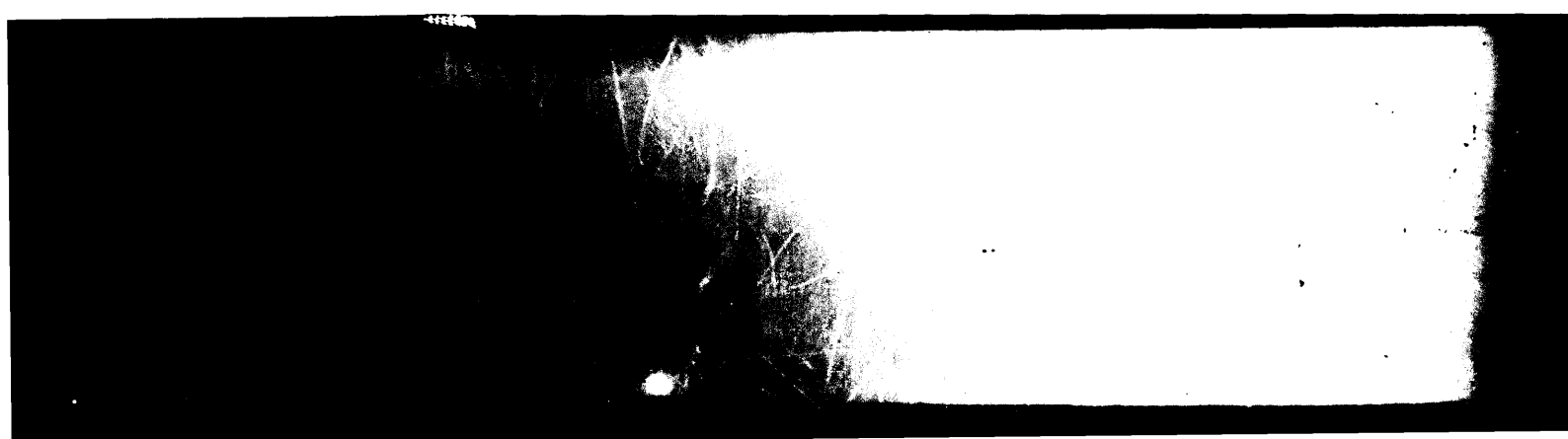
The figures indicate the lowest concentration of the compounds for which a visible growth-inhibiting effect could be observed, in comparison with the control tubes.

It must be noted, that in these series no investigations were made with concentrations $>1 : 50.000$. It is possible therefore, that inhibition of growth may occur at higher concentrations.

The dilutions of the compounds with the broth cultures were made in the usual manner.

The compounds marked with * are too sparingly soluble to give concentrations 1 : 50.000; investigations were therefore made with saturated solutions.

The yeast and the moulds were cultured on the well-known broth cultures.



The figures in Table II indicate the lowest concentrations of the compounds for which a visible growth-inhibiting effect, in comparison with the control tubes, could be observed with the Gram-positive bacteria *Streptococcus haemolyticus* and *Diplococcus pneumoniae* (type I) and with the Gram-negative *Bacillus proteus*.

No investigations were made with concentrations $>1 : 50.000$.

Investigations on the growth-inhibiting effects of the compounds on the Gram-positive *Staphylococcus aureus* and the Gram-negative *Bacillus coli* differ from those described before.

In these series experiments were made with concentrations $>1 : 50.000$, as far as possible on account of the very low solubility in water of most compounds of the quinone type.

Furthermore, culture tubes were sown with an initial inoculum varying from 1600—13.000 viable organisms; the exact number of them in each series was determined afterwards in the usual manner.

In this way the possible influence of different inoculae on the growth was eliminated.

The figures in Table III indicate the limiting concentrations of the compounds tested.

The Table on page 64 shows clearly how the dilutions have been made.

Compounds numbered 1—3 have already been tested by Oxford on *Staphylococcus aureus*.

Table IV shows the results of an investigation on the toxicity of the quinones, hydroquinones and water- or fat-soluble di-esters, by oral administration to white mice in different doses. Control mice were given orally 40 mgr „Dagénan" suspended in water.

In vitro strongly antibacterial quinones and hydroquinones proved to be also very toxic to higher organisms. The tolerable dose of such compounds for a mouse of 20 gram weight varies from 2.5—5 mgr.

The di-esters proved to be rather non-toxic. Doses of 20—40 mgr are well tolerated. However, intraperitoneal injection of 20 mgr of these di-esters, dissolved in water or arachis oil, causes death after 1—24 hours, as shown in Table V.

Finally, Table VI shows the results of an investigation of the chemotherapeutic activity of these compounds, in white mice injected with a lethal dose of pneumocci.

The drugs, dissolved or suspended in water or arachis oil, were administered by a stomach tube.

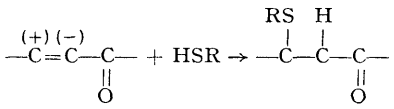
From the results of these investigations, one could conclude that, on account of their general toxic reactions and their antibacterial inactivity *in vivo*, quinones and hydroquinones which are strongly antibacterial *in vitro*, have presumably little or no therapeutic value.

The di-esters of the hydroquinones, although rather non-toxic on oral administration, appear to be also inactive *in vivo*. This conclusion is in accordance with those of Glock, Thorp, Ungar and Wien, based on their biological investigations on 4,6-dimethoxy-toluquinone, which is highly active *in vitro*.

The last chapter of this thesis contains a discussion of the relationship between chemical constitution and biological activity of a number of antibiotics, in connection with the hypothesis of Cavallito and his collaborators. As is well known, Cavallito and other investigators have shown, that several antibiotics may be inactivated by adding thiol compounds to the culture medium. This fact lead Cavallito to the hypothesis, that these antibiotics possibly act by blocking essential SH-systems of the bacterial cell.

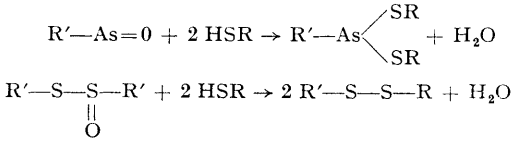
Attention has been called to the possibility that many antibiotics, as well as other antibacterial compounds, possess ,,active groupings", which may react with the SH-systems of the micro-organism. This point of view has lead us to the attractive assumption to divide those compounds in the following classes:

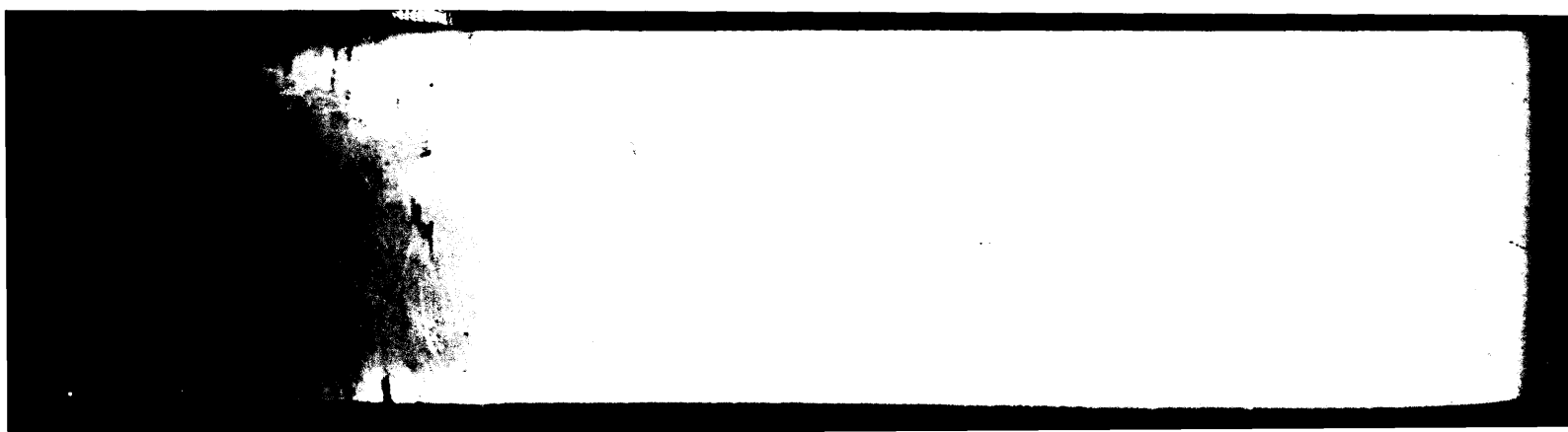
A. Compounds possessing ,,active" —C=C-groupings, that may react with thiol compounds, e.g. according the following scheme:



Possibly, the antibiotics penicillic acid, patulin, mycophenolic acid, the ,,plant antibiotic" proto-anemonine, the antibacterial compounds of the acrylophenone type, as well as some compounds possessing unsaturated lactone structures, belong to this class.

B. Compounds possessing ,,active" oxide groupings, which may react with thiol compounds, e.g. according the schemes:





Possibly, the antibiotic iodinin, the ,,plant antibiotic'' allicin and also those organic arsenic compounds, which *in vivo* after oxidation or reduction form alkylarsen oxides RAsO, belong to this class.

C. Compounds, possessing oxido-reduction systems able to oxidise SH-groups to the corresponding —S—S-compounds, e.g. compounds with a quinone structure, some dyes and the antibiotics gliotoxin and chlororaphin.

However, it must be noted, that some quinones also react with SH-compounds, through addition at the double bonds of the quinone molecule.

All these compounds may be considered as ,,substances thioloprives'', which according to Bacq (Experientia II, 349, 385 (1946)) cause characteristic phenomena in the organism, probably due to their reaction with the thiol groups of enzyme systems. Examples: H_2O_2 (oxidation of SH-groups), heavy metals (Hg, Cu etc. forming metal complexes with thiols), organic halogen compounds, such as halogen acetic acids, mustard gas, etc., in which the ,,active'' halogen atom can react with SH-groups, either by substitution or dehydrogenation.

Most compounds, mentioned under A, B and C, have been proved to be toxic for higher organisms. Therefore it appears, that these compounds are rather non-specific in reacting with SH-systems.

However, by synthesizing a large number of products with ,,active'' groupings, it may be possible to obtain compounds which might prove to be more specific in reacting with SH-systems and possibly might be of therapeutic value.

LIST OF NEW COMPOUNDS.

$C_6H_8O_9P_2Na_4$	2-methyl-5-methoxyhydroquinone tetra sodium diphosphate.
$C_8H_8O_{10}P_2Na_4$	2,6-dimethoxyhydroquinone tetra sodium diphosphate.
$C_9H_9O_4Cl$	4-chloro-3,6-dimethoxytoluquinone-2,5; m.p. 134.5—135°.
$C_9H_9O_5Cl$	3-chloro-2,5,6-trimethoxybenzoquinone-1,4; m.p. 47—49°.
$C_9H_{10}O_5$	trimethoxybenzoquinone-1,4; m.p. 160—161°.
$C_9H_{11}O_2N_2Cl$	4-chloro-3,6-bis-methylamino-toluquinone-2,5; m.p. > 360°.
$C_{11}H_{15}O_4N_2Cl$	4-chloro-3,6-bis-oxyethylamino-toluquinone-2,5; m.p. 195.5—196.5°.
$C_{11}H_{16}O_5N_2$	2-methoxy-3,6-bis-oxyethylamino-benzoquinone-1,4; m.p. 201—202°.
$C_{12}H_{14}O_5$	2-methyl-5-methoxyhydroquinone diacetate; m.p. 127—128°.
$C_{12}H_{14}O_6$	2,6-dimethoxyhydroquinone diacetate; m.p. 129—129.5°.