435. *Trypanocidal Action and Chemical Constitution. Part XIV. The Relative Velocity of Oxidation of Arylarsenoxides.*

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IT is now generally accepted by workers in chemotherapy that when arsonic acids exert a therapeutic action in protozoal diseases a major part at least of this effect is due to a direct action of tervalent arsenoxides, formed *in vivo*, on the parasites (trypanosomes, spirochetes). The main reasons which have led to this conclusion are as follows. Ehrlich found that, whereas quinquevalent arsonic acids are relatively inactive *in vitro* on trypanosomes but active *in vivo*, tervalent arsenoxides are intensely active both *in vitro* and *in vivo*. Furthermore, strains of trypanosomes which have been rendered resistant to *p*-aminophenylarsonic acid by being subjected in their animal host to sub-lethal doses of this arsenical during frequent passages from host to host exhibit a resistance *in vitro* to the reduction product *p*-aminophenylarsenoxide, suggesting that in the mammalian body this oxide must play a predominating rôle (Roehl, *Berl. klin. Woch.*, 1909, 494). Again, Terry (*J. Exp. Med.*, 1915, **21**, 258) demonstrated that when *p*-aminophenylarsonic acid is incubated with whole blood a thermostable substance is formed which is toxic to trypanosomes. Finally, Voegtlin and Smith (*J. Pharm. Exp. Ther.*, 1920, **15**, 475), from a study of the rate of disappearance of trypanosomes from the blood-stream of rats after administration of an arsonic acid (phenylglycinearsonic acid) and an arsenoxide (3-amino-4-hydroxyphenylarsenoxide), found that the latter began to act immediately, whereas the former showed a latent period of some hours before the trypanosomes began to disappear. It is therefore clear that, if reduction of arsonic acids to arsenoxides is a process involved in the mechanism of the action of arsenicals, the well-established variation of therapeutic action from one arsonic acid to another is in part due to the different speeds of

reduction of these quinquevalent arsenicals. It is of prime importance that some method should be devised for marshalling arsonic acids according to their ease or difficulty of reduction to arsenoxides under environmental conditions comparable with the biological systems, with their characteristic restricted range of hydrogen-ion concentration. In the solution of this problem definite progress has been made along two different routes, namely, the determination of (a) the oxidation–reduction potentials of arsonic acids and (b) the velocity of oxidation of arsenoxides.

If arsonic acid–arsenoxide forms a facile reversible system of the type of ferrous–ferric iron or quinone–quinol, it should be possible to determine the oxidation–reduction potential of such a system, as has been done for a series of dyes by Mansfield Clark and collaborators and for quinones by Conant and Fieser. It would then be practicable to arrange a series of arsonic acids in the order of their oxidation–reduction potentials, those of low reduction potential being more difficult to reduce than those of high reduction potential, the hydrogen electrode here being considered as of zero potential.

In order to test this possibility, some years ago, Dr. Gough and one of the present authors examined the effect of adding a series of reduction potential indicators, such as those devised by Mansfield Clark, in the reduced form, to various arsonic acids in buffered solutions, the whole operation being carried out in a hydrogen atmosphere. In no case was the oxidised form of the indicator dye produced. This seemed to indicate either that the arsonic acid–arsenoxide system was not a reversible system like the quinone system, or that the arsonic acids tested had very low reduction potentials, outside the range covered by the indicator dyes. The former view seemed to us at that time to be the more probable. In the meantime Baranger (Thesis, Paris, 1931; *Bull. Soc. chim.*, 1932, **51**, 203) has made an electrochemical study of arsonic acids, but could not determine their reduction potentials by the method of titration; that is to say, no facile equilibrium of oxidised and reduced forms was obtained by approaching the system from the arsonic acid side with a reducing agent, or from the arsenoxide side with an oxidising agent. The method of mixtures did, however, appear to give definitely reproducible potentials, and of the logarithmic form required by theory. This method is, however, at present restricted in scope, since it is carried out at p_H 8·9, a degree of alkalinity only sufficient to dissolve arsenoxides containing a phenolic group.

Another independent method of ranging arsonic acids would be a determination of their relative velocities of reduction with a common reducing agent. The experimental difficulties here are

very great; the choice of reducing agents is very limited and the reaction proceeds in a number of stages, *viz.*,

$$R{\cdot}AsO_3H_2 \longrightarrow R{\cdot}AsO \longrightarrow RAs{:}AsR \longrightarrow R{\cdot}AsH_2$$

The converse, however, is not open to the same objection, since if the arsenoxides were chosen they could only be oxidised to the arsonic acids. Here again the selection of a suitable oxidising agent, the proportion of which can be estimated quantitatively with time, raises difficulties which have been eventually overcome by the use of the optically active amino-acid cystine. This substance oxidises arsenoxides in alkaline solution to arsonic acids (Cohen, King, and Strangeways, J., 1931, 3048), the specific rotation for the mercury green line changing from about $-104°$ to $-3°$ at room temperature as cystine becomes reduced to cysteine:

$$R{\cdot}As\begin{matrix}OH\\OH\end{matrix} + \begin{matrix}S{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H\\|\\S{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H\end{matrix} \xrightarrow{OH}$$

$$R{\cdot}AsO_2 + 2SH{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$$

It has thus been possible to determine the bimolecular velocity constants of a series of arsenoxides, and so to arrange them in the order of their ease of oxidation. The assumption that the reverse order should correspond to the ease of reduction of the corresponding arsonic acids, we believe to be justified from the following considerations. If the reduction potential be determined for a series of quinones with various substituents, then a quinone which is reduced with difficulty, *i.e.*, one of low reduction potential, will be the oxidised form of a quinol which is easily oxidised, and quinones more readily reduced will correspond to quinols which are less readily oxidised. In fact, in such facile reversible systems as can be represented on the quinone–quinol model the sequence of increasing ease of reduction of the quinones is necessarily the sequence of decreasing ease of oxidation of the corresponding quinols. If, then, it be admitted that Baranger's measurements of reduction potential justify the classification of the arsonic acid–arsenoxide system, with its admitted unique peculiarities, as belonging to the quinone–quinol type of reversible system, then, as a working hypothesis, environmental factors being ignored, it seems reasonable to assume that the increasing order of velocities of oxidation of a series of arsenoxides towards a common oxidising agent is the reverse of the order of the velocities of reduction of the corresponding arsonic acids.

Kinetic Measurements.

Preliminary expts. on benzamide-*p*-arsenoxide and cystine having indicated the most suitable concn. of reactants and alkali for following the course of the

reaction, the following conditions were used throughout. Cystine (0·120 g.; 0·005 mol.) was dissolved in 25 c.c. of 0·2*N*-NaOH and, as a rule, the calc. mol. equiv. of the arylarsenoxide was similarly dissolved. When the dichloroarsine was more readily available as a cryst. material, it was dissolved in the calc. amount of 0·5*N*-NaOH and the vol. made up to 25 c.c. with H_2O, so that the final concn. of alkali was the same as in the expts. using free arsenoxides. Equal vols. of the clarified solutions were mixed at 20°, and rapidly transferred to a 4 dcm. Schmidt and Haensch polarimeter tube, completely jacketed and maintained at 20° by H_2O circulated from a thermostat tank. The rotation (λ = 5461 Å.) of the mixture was observed at intervals, the values obtained were converted into sp. rotations on the basis of the original cystine content of the mixture, and the vel. constants were calculated from the bimolecular equation $k = x/at(a - x)$, in which x is the change in sp. rotation in the time t (min.), and a, being proportional to the original cystine content, is the difference between the sp. rotations of cystine and cysteine. The stock of cystine had been repeatedly pptd. and had $[\alpha]_{5461}^{20°}$ − 103·7° (c = 0·24 in 0·2*N*-NaOH) and a specimen of cysteine carefully prepared from this cystine had $[\alpha]_{5461}^{20°}$ − 2·7°. Thus a = − 101 and x = −103·7 less the sp. rotation at time t. All the expts. were done in duplicate, some in triplicate, and control expts. on cystine in alkali showed that the rotation was unchanged for 3 hr., a period in excess of that over which observations were made in any expts.

The results are in the following tables, two typical examples being recorded in full to show the variations observed in k.

I. Phenylglycineamide-*p*-arsenoxide.

t (min.).	x.	$k \times 10^5$.
6	15·15	29·11
10	24·0	30·85
15	31·3	29·64
20	39·1	31·27
25	43·3	29·72
30	49·54	31·77
40	54·74	29·30
	Mean	30·24

II. Phenol-*p*-arsenoxide.

t (min.).	x.	$k \times 10^5$.
5	8·4	17·98
10	15·2	17·54
20	24·0	15·43
25	30·3	16·96
30	35·5	17·89
35	38·1	17·13
40	40·67	16·69
50	44·33	15·5
60	48·5	15·24
	Mean	16·71

TABLE A.

Substituent.	$k \times 10^5$ *a*.	*b*.	Mean.	Tol.*
p-NHAc	38·52	38·56	38·54	125
p-CO·NH_2	34·33	34·56	34·45	100
H	32·71	32·02	32·37	2·5
p-NH·CH_2·CO·NH_2	30·24	30·65	30·45	200
p-OMe	30·0	30·1	30·05	0·75
p-Cl	25·23	24·85	25·04	0·75
p-CO_2H	22·48	22·77	22·62	20
p-SO_2·NH_2	22·29	22·56	22·43	50
3-NH_2 : 4-OH	19·58	20·22	19·9	100
p-OH	16·68	16·71	16·7	20
4-NHAc : 2-OH	2·17	2·17	2·17	100
o-OH	2·87	2·76	2·82	—

* No great accuracy is claimed for these values such as would be attained by the use of large numbers of mice, followed by a statistical treatment of the results. They are the max. doses tolerated by at least 4 out of 5 mice for 7 days, the drug being administered intravenously.

The variation of k with the constitution of the arsenoxide is shown in the Table A, in which the last col. shows the max. tolerated dose for mice, of the corresponding quinquevalent arsonic acid, expressed in mg. per 100 g. of mouse. The toxicities are proportional to the reciprocals of these figures.

Discussion of Results.

The oxidation of phenylarsenoxide to the arsonic acid, expressed electronically, consists in the co-ordination of an oxygen atom through the lone pair of electrons of the tervalent arsenic atom. If a substituent group X is present which attracts electrons and so lowers the electron pressure over the benzene nucleus, the effect will be transmitted to the arsenic atom, which will be restrained in co-ordinating oxygen, thus giving rise to a smaller velocity constant than for unsubstituted phenylarsenoxide. Conversely an electron-repelling group will enhance the tendency of the arsenic to add on oxygen, and this will be shown by a higher velocity constant.

The same conclusions follow if we adopt Baranger's quantitative electronic representation of the reduction of an arsonic acid, $R{\cdot}AsO^{++} + 2e \longrightarrow R{\cdot}AsO$, where $R{\cdot}AsO^{++}$ is a hypothetical bivalent kation of an arsonic acid.

The most complete standard of reference of the inductive effect of substituent groups is that of the ionisation constants of m- and p-substituted benzoic acids as shown in the following table.

Ionisation Constants of m- *and* p-*Substituted Benzoic Acids.**

	NO_2.	CN.	$SO_2{\cdot}NH_2$.	CO_2H.	Cl.	OAc.
p	40·1	31	26	13	9·3	8·9
m	34·8	19·9	—	29	15·5	13·1
	H.	NHAc.	Me.	OMe.	OH.	NH_2.
p	6·6	5·2	4·5	3·2	2·9	1·2
m	6·6	8·5	5·6	—	8·7	1·67

* Compiled from the Landolt–Bornstein "Tabellen"; and Lucas and Valby, *J. Amer. Chem. Soc.*, 1929, **51**, 2718.

With two exceptions the sequence of inductive effects of the substituent groups is the same whether the substituent is in the p- or the m-position to the carboxyl group. For m- and p-substituted arsenoxides one would therefore expect that the higher the ionisation constant of the corresponding substituted benzoic acids, the slower the velocity of oxidation of the arsenoxide to the arsonic acid. Comparison of the two tables of velocity constants and ionisation constants shows that the groups $SO_2{\cdot}NH_2$, CO_2H, Cl, H, and NHAc follow the correct sequence in both tables, but OMe and particularly p-OH are anomalous. For these groups we are not able to offer any explanation. The result with p-OH is, however, in agreement with Baranger's observation that phenol-p-arsonic acid is readily re-

duced. It should be emphasised that, whereas the inductive effects of different groups as determined by ionisation constants, dipole measurements, or reduction potential measurements of substituted quinones, are those of the normal molecule, the inductive effects as deduced from the velocity of oxidation of the arsenoxides are determined in an alkaline environment, where groups such as $SO_2{\cdot}NH_2$, CO_2H, $CO{\cdot}NH_2$, and OH will exist to a greater or less extent as ionised forms conferring their own velocities of reaction. Such a possibility does not, however, overcome the anomaly of the *p*-substituted hydroxyl group. *o*-Phenolarsenoxide and 4-acetamido-2-hydroxyphenylarsenoxide have very low velocities of oxidation and it seems probable that here steric factors intervene.

There remains for consideration the bearing of these results on the biological properties of these substances. When primary arsonic acids are reduced to arsenoxides the toxicity to the mammal is increased manyfold. Since, moreover, arsonic acids must undergo reduction in mammalian tissues before they can manifest their curative action on the parasites present, it seems reasonable to assume that, other factors being the same, the toxicity of arsonic acids to the host may be a measure of the amount of reduction by the mammalian tissues. Other factors are not actually the same, since the arsenoxides corresponding to this series of acids, if directly administered, are not equal in toxicity. We should, however, expect to find some indication of correspondence between ease of reduction of arsonic acids and toxicity. Examination of the column showing the doses tolerated by mice, of a series of arsonic acids and that of the velocities of oxidation of the corresponding arsenoxides, in Table A, shows that there is no such relationship discernible between these properties. It seems probable that other factors intervene which mask the anticipated relationship in addition to the varying toxicities of the arsenoxides already mentioned. Of these other factors there is the rate of excretion, for it has been shown by Voegtlin and Thompson (*J. Pharm. Exp. Ther.*, 1922, **20**, 91), in the case of certain *p*-substituted arsonic acids studied in the rat, that over 80% is excreted through the kidneys within 6 hours. The amount of an arsonic acid available for reduction in the tissues to the arsenoxide can thus be but a small fraction of the arsonic acid administered. Ehrlich, for instance, showed that phenol-*p*-arsenoxide was 173 times as toxic to mice as phenol-*p*-arsonic acid. If the whole of the toxicity of phenol-*p*-arsonic acid could be attributed to the fraction reduced to the arsenoxide by the mammalian tissues, the percentage reduction can only have been of the order of 0·5%. In such circumstances it is not surprising that the expected relationship between the velocity constants of

oxidation of the arsenoxides and the toxicities of the corresponding arsonic acids is not observed. Still less would one expect to find any relation between the ease of reduction of arsonic acids and their curative actions such as Baranger sought to find, since curative action is a toxicity to trypanosomes measurable only within the limits of non-toxicity of the arsenical to the mammalian host. Neither from toxicity for the mammal, nor from the curative action on an infection can we make any deduction as to the rate at which the compound is reduced in the tissues. This rate can, at best, be only one of many factors determining activity in either direction.

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