CCCCXXI.—*Trypanocidal Action and Chemical Constitution. Part X. Arylthioarsinites.*

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Historical.

The thioarsinites are a group of substances in which tervalent arsenic is linked through sulphur to one, two, or three organic radicals. Within the last decade they have been the subject of numerous investigations on account of their potential therapeutic importance, but a study of the literature of the subject reveals many anomalies.

The first thioarsinite to be prepared appears to be arsenic trithiolacetic acid, $As(S{\cdot}CH_2{\cdot}CO_2H)_3$, a crystalline substance forming sodium salts, prepared from arsenious oxide and thiolacetic acid in aqueous solution by Rosenheim and Davidsohn (*Z. anorg. Chem.*, 1904, **41**, 231). This was followed by Friedberger's observation (*Berl. klin. Woch.*, 1908, 1714) that *p*-aminophenylarsonic acid when mixed with thiolacetic acid became highly toxic to mice and contained an effective trypanocidal agent. According to experiments made by Bertheim (quoted by Roehl, *Berl. klin. Woch.*, 1909, 494) *p*-aminophenylarsenoxide is produced by reduction and this, by analogy with arsenious oxide, combines with excess of thiolacetic acid to form crystalline di(carboxymethyl) 4-aminophenylthioarsinite:

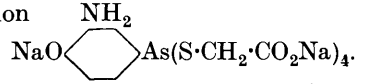
$$R{\cdot}AsO_3H_2 \xrightarrow{SH{\cdot}CH_2{\cdot}CO_2H} R{\cdot}AsO \xrightarrow{SH{\cdot}CH_2{\cdot}CO_2H} R{\cdot}As\begin{matrix}S{\cdot}CH_2{\cdot}CO_2H\\S{\cdot}CH_2{\cdot}CO_2H\end{matrix}$$

The subject then remained in abeyance until Voegtlin, Dyer, and Leonard (*U.S. Public Health Rep.*, 1923, **38**, 1911), in an addendum to their paper, described the preparation of an analogously constituted compound, di(carboxymethyl) 3-amino-4-hydroxyphenylthioarsinite, by heating together 3-amino-4-hydroxyphenylarsenoxide hydrochloride and 4 molecular proportions of thiolacetic acid. It is stated that the compound showed a marked delay in trypanocidal action as compared with the equimolecular proportion of the parent arsenoxide. The potential importance of such substances was grasped by Kharasch, who covered by patent (U.S.P. 1,589,599/1924) the production of water-soluble organo-metallic compounds from compounds of mercury, arsenic, antimony, and bismuth and as an example described the preparation of di(carboxymethyl) phenylthioarsinite by the addition of potassium thiolacetate to an alcoholic solution of phenyldichloroarsine:

$$Ph{\cdot}AsCl_2 + 2SH{\cdot}CH_2{\cdot}CO_2K \longrightarrow Ph{\cdot}As(S{\cdot}CH_2{\cdot}CO_2H)_2.$$

This patent was considerably amplified by its author in its application to arsenicals in a fresh publication (U.S.P. 1,677,392/1927). It was there pointed out that tervalent arsenicals are of limited application, since they are insoluble in water and, being very weak acids, require a strong alkali for their solution. Such oxides become effectively available for uses similar to those of quinquevalent arsenicals by condensation with thiol compounds containing carboxyl or sulphogroups. A number of thioarsinites were described, substances such as *o*-thiolbenzoic acid, *p*-thiolbenzenesulphonic acid, *p*-thiolphenylacetic acid, cysteine, and β-thiolpropionic acid being used as sources of thiol groups. The compounds are said to be less toxic than would be expected from their content of arylarsenoxide. A little

later in the same year Poulenc Frères and Oechslin (F.P. 643,911) patented similar thioarsinites from thiolacetamide, β-thiolethyl alcohol, potassium xanthate, and the monothiol substitution product of glycerol. In the following year Parke Davis and Co. were granted a patent (E.P. 331,195) which made some remarkable claims. It stated that by causing arsonic acids to react with thiol compounds new quinquevalent derivatives are formed with an increased toxicity to spirochaetes. This was considered novel, since Voegtlin, Dyer, and Leonard's observation was that, by using tervalent arsenicals in the same way, compounds are obtained whose toxicity to spirochaetes is less than that of the arsenious acids from which they are derived. The preparation of a variety of types is described; for instance, 3-amino-4-hydroxyphenylarsonic acid is dissolved in an aqueous solution of 4 molecular proportions of sodium thiolacetate, the solution warmed, made just alkaline with sodium hydroxide, and the substance precipitated by pouring into methyl alcohol, followed by addition of ether. To this substance is attributed the constitution



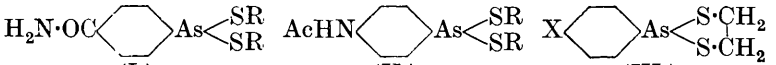
In other cases analogous compounds were said to have been obtained by evaporation of the solutions to dryness. If only two molecular proportions of sodium thiolacetate were used, compounds of the type $R{\cdot}AsO(S{\cdot}CH_2{\cdot}CO_2Na)_2$ were likewise said to have been produced. Early in 1929 Labes (*Arch. exp. Path. Pharm.*, **141**, 159) showed that cysteine and arsenious oxide combine to give a well-crystallised but very sparingly soluble substance, tricysteinylarsine, and later in the same year Barber and May and Baker, Ltd., were granted a patent (E.P. 331,869) which claimed a process for preparing substituted thioarsinites by treating one molecular proportion of an arsonic acid with 4 molecular proportions of a thiol compound, two of the latter being used to reduce the arsonic acid to the arsenoxide, which then condenses *in situ* with the 2 remaining molecular proportions of free thiol compound. This method, it will be recalled, had already been envisaged if not employed by Bertheim. The thiol compounds used in this specification were thiolacetic acid, its amide and ethyl ester, cysteine, and β-thiolethyl alcohol. The subject matter of this patent with certain amplifications was published later by Barber (J., 1929, 1020). The same author also introduced thiolacetamide (*ibid.*, p. 1024) as a valuable reagent for the characterisation of arsonic acids, and illustrated its use in subsequent papers (*ibid.*, p. 2333; 1930, 2047). Binz, Räth, and Maier-Bode (*Annalen*, 1930, **478**, 22) described the use of thiophenol for the same purpose. In 1930 Gough and King (J., 673)

described the preparation and properties of di(carboxymethyl) benzamide-*p*-thioarsinite and criticised some of the views expressed by Barber. Later in the same year Johnson and Voegtlin described the preparation (*J. Biol. Chem.*, 1930, **89**, 27) of the thioarsinites from cysteine and 3-amino-4-hydroxyphenylarsenoxide and from arsenious acid, both of which compounds had been previously prepared, the former by Barber, the latter by Labes.

Present Investigation.

In the present investigation a number of dithioarsinites capable of forming water-soluble sodium salts have been prepared by direct condensation of arylarsenious oxides with two molecular proportions of a thiol compound containing a carboxyl group. The reaction is carried out in aqueous or sodium hydrogen carbonate solution, whichever is more suitable to the particular thiol compound, and its progress is marked by the rapid dissolution of the otherwise sparingly soluble arsenoxide. Although this method necessitates the prior preparation of the tervalent arsenical, it possesses the advantage of yielding a single product. The yields are in every case excellent and the products are easily purified.

Starting with benzamide-*p*-arsenoxide and acetanilide-*p*-arsenoxide as parent arsenicals, two series of compounds, (I) and (II), have been prepared by employing in the condensations α-thiolacetic



(I.) (II.) (III.)

acid, β-thiolpropionic acid and its α-amino-derivative, the naturally occurring amino-acid cysteine, *o*-thiolbenzoic acid, *m*-thiolbenzoic acid, α-thiolacetamide, and the biologically important tripeptide, glutathione. It was thought that the preparation and study of two such parallel series of compounds would throw some light on their mode of action and their therapeutic possibilities.

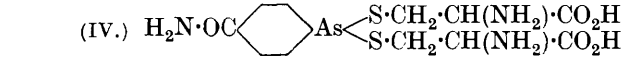
Finally, dithiolethane has yielded representatives of the novel type (III). The influence of the heterocyclic environment of the arsenic atom in compounds of this type is shown by their solubility in common organic solvents, a property unusual among arsenicals generally.

The hydrolysis of the thioarsinites has already been the subject of discussion. Barber (J., 1929, 1021) held that hydrolysis was not effected by alkali and that the intact thioarsinites gave an intense nitroprusside reaction, while in an earlier communication of this series (Gough and King, *loc. cit.*) the view was expressed that in weakly alkaline and even in aqueous solution there was at least partial hydrolysis into the constituent arsenoxide and thiol com-

pound. The present work has shown that a modified form of the latter view correctly expresses the stability of the arsenic–sulphur linkage. Sodium hydrogen carbonate solutions of all the purified compounds of types (I) and (II) so far examined and derived from aliphatic thiol compounds have either failed to give a nitroprusside reaction or have given a very weak coloration, whereas the aliphatic thiol compounds from which they were derived all give an intense coloration under parallel conditions. This characteristic coloration is, however, immediately and intensely developed by dissolving the thioarsinites in cold *N*/10-sodium hydroxide solution. It is therefore suggested that in solutions of thioarsinites the intact molecule is in equilibrium with its hydrolytic products; considerable hydrolysis is effected by caustic alkali and very little or no hydrolysis effected by sodium hydrogen carbonate.

$$R\cdot As\langle^{SR'}_{SR'} + 2H_2O \underset{H^{\cdot}}{\overset{OH'}{\rightleftharpoons}} R\cdot As(OH)_2 + 2R'\cdot SH \quad . \quad . \quad (i)$$

Further support for this view is forthcoming from polarimetric observations on a suitable thioarsinite. The following table gives the specific rotations for the mercury green line, $[\alpha]^{20°}_{5461}$, $c = 1$, in *N*-hydrochloric acid, *N*-sodium hydroxide, and saturated sodium

(IV.) 

hydrogen carbonate solution for cysteine, the corresponding disulphide cystine, and for the dithioarsinite (IV) derived from benzamide-*p*-arsenoxide.

	N-HCl.	Saturated $NaHCO_3$ soln.	*N*-NaOH.
Cystine	−259·5°	—	−100·2°
Cysteine	+ 11·4	+15·9°	− 2·7
Thioarsinite	+ 12·9	+43·1	− 1·8
	(+ 23·2)	(+77·4)	(− 3·2)

The figures in parentheses for the thioarsinite are calculated on the cysteine content on the assumption that the whole of the rotation is due to free cysteine. It is at once obvious that in *N*-hydrochloric acid and in saturated sodium hydrogen carbonate solution the thioarsinite has its own characteristic specific rotation, whereas in sodium hydroxide the rotation falls immediately to a value which, calculated on the cysteine content, indicates a more or less complete hydrolysis to free cysteine.

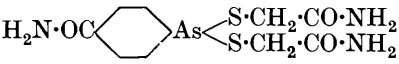
The same result is shown by approaching the problem from the synthetic side. The specific rotation of a 2·4% solution of cysteine in *N*-sodium hydroxide is recorded in the first column in the following table. Two samples of this solution were taken. The first was diluted with an equal volume of a *N*-sodium hydroxide solution

of the calculated amount of benzamide-*p*-arsenoxide required for condensation with the cysteine, the second sample was diluted with an equal volume of *N*-sodium hydroxide and thus served as a control.

Cysteine in *N*-NaOH.	Cysteine + arsenoxide in *N*-NaOH.	Cysteine + *N*-NaOH in *N*-NaOH.
−2·9°	−3·4°	−3·5°

It will be observed that the small negative rotation of cysteine is, within the limits of experimental error, unchanged by addition of the arsenoxide.

Further support for these views is forthcoming from the instructive behaviour of di(carbamylmethyl) benzamide-*p*-thioarsinite (V).



(V.)

Although the molecule of this compound contains no apparent acidic centre, it dissolves readily in cold dilute alkali solution with development of a strong nitroprusside reaction. This can only be interpreted by hydrolytic fission of the thioarsinite group with production of acidic constituents. When the alkaline solution is saturated with carbon dioxide, the parent substance is reconstituted and crystallises from the solution, being insoluble in sodium hydrogen carbonate solution. The ease of resynthesis of this and other thioarsinites explains the failure to isolate the products of hydrolysis. To achieve this would involve acidification, an operation which immediately displaces the equilibrium (equation i) in favour of the formation of thioarsinite. Similarly it has not been found possible to show, by recovery of starting materials, that arsenoxide and thiol compound do not condense in alkaline solution.

In further elucidation of the chemistry of these interesting substances, the highly significant observation has been made that an alkaline solution of cystine (disulphide form), when treated with benzamide-*p*-arsenoxide, immediately develops an intense nitroprusside reaction in the cold, indicating the presence of cysteine

$$\mathrm{R{\cdot}AsO} + \begin{matrix}\mathrm{SR'}\\ |\\ \mathrm{SR'}\end{matrix} + 2\mathrm{H_2O} \xrightarrow{\mathrm{OH'}} \mathrm{R{\cdot}AsO_3H_2} + 2\mathrm{HS{\cdot}R'}$$

(thiol form). The same result has been obtained with a variety of arsenoxides and also with disulphidoacetic acid and disulphidopropionic acid in addition to cystine. The reaction is in fact of value as a qualitative test in the chemistry of aliphatic thiol compounds and disulphides.

Observations of the optical rotation of cystine with an arsenoxide fully bear out these conclusions. The specific rotation of cystine in *N*-sodium hydroxide ($c = 1$) is given in the following table. The

solution used was divided, one portion being diluted with an equal volume of *N*-sodium hydroxide to act as a control, and the other diluted with an equal volume of *N*-sodium hydroxide containing an equimolecular proportion of benzamide-*p*-arsenoxide. The figures for $[\alpha]_{5461}^{20^\circ}$ were as follows :

Cystine in *N*-NaOH	−100·5°
Cystine in *N*-NaOH diluted with *N*-NaOH	− 97·4
Cystine diluted with arsenoxide in *N*-NaOH ...	− 9·9

The rotation of cystine in the presence of the arsenoxide is in complete accord with its conversion to a large extent into cysteine. In alkaline solution, therefore, arsenoxide and disulphide are converted into the more stable system, arsonic acid and thiol compound, whence it must be concluded that thiol compounds do not materially reduce arsonic acids in alkaline solution.

The results so far obtained necessitate the revision of current views. The inability of thiol compounds and arsenoxides to condense and of thiol compounds to reduce arsonic acids in alkaline solution invalidates the mechanism proposed for the reaction between the latter (Barber, J., 1929, 1021). We suggest that in solutions bordering on neutrality an arsonic acid condenses with four molecular proportions of a thiol compound, forming an intermediate tetrathioarsonate. In the second step of the reaction, two thioalkyl (or thioaryl) groups split off from the unstable molecule and link with each other, resulting in the formation of a dithioarsinite and disulphide :

$$R\cdot AsO(OH)_2 + 4HS\cdot R' \longrightarrow R\cdot As(SR')_4 \longrightarrow R\cdot As(SR')_2 + (\cdot SR')_2$$

Attempts to realise the intermediate tetrathioarsonates have all failed, as have attempts to add on a sulphur atom to the thioarsinites with formation of thioarsonates of the type $R\cdot AsS(SR')_2$. It naturally follows that the tetrathioarsonates described by Parke Davis and Co. (*loc. cit.*) are in fact equimolecular mixtures of thioarsinite and disulphide, as their mode of isolation would suggest.

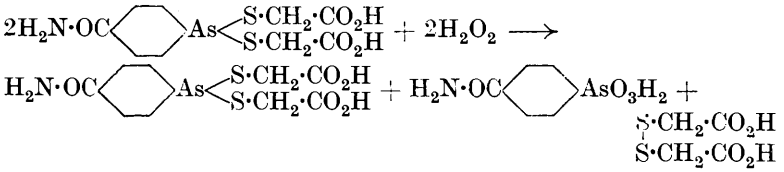
The adoption of the above views on the nature of the thioarsinites and the oxidation–reduction relationships between arsenic and sulphur compounds leads to a rational interpretation of all the known reactions of these compounds and makes possible the prediction of the reaction between arseno-compounds and disulphides. By choice of suitable compounds it has been shown that two molecules of a disulphide are reduced by an arseno-compound in alkaline solution, and neutralisation leads to the formation of a thioarsinite :

$$\begin{matrix} R\cdot As \\ R\cdot As \end{matrix} + 2\begin{matrix} SR' \\ SR' \end{matrix} \longrightarrow 2R\cdot As\begin{matrix} SR' \\ SR' \end{matrix}$$

Thus, by using 4 : 4′-arsenobenzoic acid and cystine, the thio-

arsinite has been prepared which is identical with the product of the condensation of *p*-benzarsenoxide and cysteine.

Lastly, the behaviour of dithioarsinites on partial oxidation is in accord with the principles discussed above. The oxidation of di(carboxymethyl) benzamide-*p*-thioarsinite by one molecular proportion of hydrogen peroxide in sodium hydrogen carbonate solution has already been described by Gough and King (*loc. cit.*). Their result has been confirmed, namely, that unchanged dithioarsinite and benzamide-*p*-arsonic acid are formed, and has been amplified by the isolation of the remaining constituent, disulphidoacetic acid :



To what extent hydrogen peroxide attacks the intact thioarsinite molecule in preference to its hydrolytic products expressed by equation (i) is a debatable point, but whatever the intermediate mechanism, it follows from the principles that have been previously discussed and established that the final products, in sodium hydrogen carbonate solution, will be such as are actually found. In alkaline solution, where the thioarsinites are completely hydrolysed, oxidation would necessarily be confined to the arsenoxide.

Therapeutic Results and Discussion.

The two parallel series of thioarsinites described in this communication have been tested for trypanocidal activity, on an experimental infection of *Trypanosoma equiperdum* in mice. A summary of the results is recorded in the following table, *T* signifying the maximum dose tolerated, expressed in milligrams per gram of mouse, *C* the minimum curative dose, and *r* the number of days during which the blood-stream remained free from trypanosomes. The figures in parentheses are the molecular weights of the constituents from which the dithioarsinites are built up.

Dithioarsinites, $R{\cdot}As{<}^{SR'}_{SR'}$.

Thiol constituent.	Benzamide-*p*-arsenoxide (211).			Acetanilide-*p*-arsenoxide (225).		
	T.	*C*.	*r*.	*T*.	*C*.	*r*.
—	0·03	0·01	14	0·02	0·005	>30
Thiolacetic acid (76)	0·05	0·03	>30	0·05	0·01	>30
β-Thiolpropionic acid (88) ...	0·075	0·01	>30	0·05	0·005	>30
Cysteine (121)	0·1	0·01	>30	0·05	0·0075	>30
o-Thiolbenzoic acid (154) ...	0·075	0·01	>30	0·04	0·02	>30
m-Thiolbenzoic acid (154) ...	0·1	0·025	>30	0·1	0·01	>30
Glutathione (307)	0·35	0·1	19	0·2	0·075	11

A careful examination of the table shows that, as the molecular weight of the thiol constituent increases, with one or two exceptions, the maximum tolerated dose, T, also increases, *i.e.*, the thioarsinites become less toxic. The decreased toxicity, however, is greater than that calculated from the percentage content of the toxic arsenoxide, indicating that these thioarsinites when injected into the mammalian blood-stream as neutral sodium salts do persist as molecular units for some time at least. This agrees with the statement of Kharasch (*loc. cit.*). It is thus possible to administer an amount of potential arsenoxide greater than that represented by the maximum tolerated dose of the free arsenoxide. The great majority of these dithioarsinites can also produce "permanent cures" in infected mice, on smaller fractions of the tolerated doses than can the parent arsenoxides, as shown by no reappearance of trypanosomes in the blood of mice over a period of 30 days. These compounds therefore possess the double advantage over the parent arsenoxides of forming neutral soluble sodium salts and of exhibiting increased therapeutic efficiency. Although permanent in their effect, these substances are transitory in action, since, like arsonic acids and unlike arseno-compounds, they have no influence on an infection given 24 hours after the drug. By reason of their initial stability at the p_H of the blood-stream, they are distributed throughout the whole circulatory system and there gradually liberate by some mechanism, hydrolytic or otherwise, their toxic oxides.

It is interesting to contrast the properties of the dithioarsinites prepared from the amino-acid, cysteine, with those prepared from the tripeptide, glutathione, which contains cysteine, glutamic acid, and glycine. Glutathione is very soluble in water and if a sparingly soluble arsenoxide, such as acetanilide-*p*-arsenoxide, is suspended in the aqueous solution at room temperature it is taken up until the nitroprusside reaction for the free thiol group becomes negative. The dithioarsinites so formed are also very soluble in water. On the other hand, the corresponding dithioarsinites from cysteine are not soluble in water to any comparable extent and their sodium salts, particularly that from benzamide-*p*-arsenoxide and cysteine, are not readily soluble in water. It is possibly, therefore, the high aqueous solubility of the glutathione derivatives of the arsenoxides which accounts for their low therapeutic efficacy, since they will be too readily excreted from the mammalian system.

It has been shown by Rosenthal and Voegtlin (*J. Pharm. Exp. Ther.*, 1930, **39**, 347) that glutathione has the property of inhibiting the toxic action of 3-amino-4-hydroxyphenylarsenoxide on rats and on trypanosomes *in vitro*, if given in the proportion of 10 molecules of glutathione to one of arsenoxide. It is evident that glutathione

combines with the arsenoxide, forming a dithioarsinite, the excess of glutathione reducing the dissociation of the complex to a minimum. This incidentally affords further evidence for the view expressed by Gough and King (*loc. cit.*) that the toxic action of thioarsinites for trypanosomes is not a property of the intact molecule but of the arsenoxide produced by hydrolysis.

Finally, what light do these results throw on the mechanism of the lethal action of arsenicals on trypanosomes? Ehrlich (*Ber.*, 1909, **42**, 42), impressed by Heffter's observations on the thiol constituents of tissues, suggested that the toxic action of arsenicals might lie in their affinity for thiol groups. This view has been adopted and amplified by Voegtlin and his associates in numerous scientific communications. They regard glutathione as the main arsenic-receptor and consider that the immobilisation of the glutathione in this way interferes with the respiratory mechanism of living cells. Levaditi, Anderson, and Manin (*Bull. Soc. Path. Expt.*, 1928, **21**, 676), whilst recognising that glutathione may play a part, prefer a more general statement that the element sulphur is of prime importance in the pharmacodynamics of arsenicals. Warburg, on the other hand, believes that the inhibitory effect of arsenic on tissue respiration is due to a specific chemical combination with the iron of the respiratory ferment. Our own view is that the remarkable affinity between arsenic and organically bound sulphur established by the experiments described in this communication fully justifies the hypothesis that the lethal action of arsenic on living tissues is a chemical action, and may very well be an action on thiol groups, and possibly on glutathione in particular. That such temporary immobilisation of thiol structures might bring about the death of the living cell by interfering with some link in the chain of metabolic processes is also possible, but it would be premature to attribute this action to a direct effect on respiration controlled by glutathione, as there is no consensus of opinion at the present time on the real function of glutathione in living processes.

EXPERIMENTAL.

Acetanilide-p-*arsenoxide*.—Through a solution of 4-acetamidophenylarsonic acid monohydrate (22·2 g.) in alcohol (200 c.c.) and hydrochloric acid (16 c.c.; *d* 1·16) containing a trace of potassium iodide, sulphur dioxide was passed for 2 hours at 0° (compare Bertheim, *Ber.*, 1911, **44**, 1073). An equal volume of water was added, and the solution resaturated. A further 500 c.c. of water were added, and the solution again saturated with sulphur dioxide and kept at 0° for 3 days. The solid which separated was collected, washed with cold *N*-hydrochloric acid and with water, and finally

triturated with sodium hydrogen carbonate solution and dried. Yield, 15·35 g.; m. p. 285—288° (Found: As, 33·7. Calc.: As, 33·3%).

Benzamide-p-*arsenoxide*.—The procedure of Gough and King (*loc. cit.*) was followed for the preparation of this compound.

Dithioarsinites derived from Acetanilide-p-*arsenoxide*.—*Di*(*carboxymethyl*) *acetanilide*-p-*thioarsinite*. The parent arsenoxide (3 g.) was added to a solution of α-thiolacetic acid (1·7 g.) in water (40 c.c.). Solution rapidly ensued when the mixture was heated. On cooling, a clear colourless oil separated, which was reprecipitated from a solution of its sodium salt in small leaflets (3·5 g.), m. p. 108—110° [Found: *M*, by iodine titration (Barber, *loc. cit.*), 388·8. $C_{12}H_{14}O_5NS_2As$ requires *M*, 391]. The nitroprusside reaction of this compound is very feeble in sodium hydrogen carbonate solution, and very intense in cold 0·1*N*-sodium hydroxide.

Di(β-*carboxyethyl*) *acetanilide*-p-*thioarsinite*. The parent arsenoxide (2·3 g.) was added to a solution of β-thiolpropionic acid (2·1 g.) in water (50 c.c.) and sodium hydrogen carbonate (1·68 g.). After boiling for 5 minutes, the almost clear solution was filtered and made acid to Congo-paper. The product (3·95 g.) crystallised from 38 parts of boiling water in filmy diamonds, m. p. 147° (Found: As, 17·7. $C_{14}H_{18}O_5NS_2As$ requires As, 17·9%). In sodium hydrogen carbonate solution, the nitroprusside reaction is barely visible.

Di(β-*carboxy*-β-*aminoethyl*) *acetanilide*-p-*thioarsinite*. A mixture of cysteine hydrochloride (1·75 g.), water (25 c.c.), sodium hydrogen carbonate (1·5 g.), and the parent arsenoxide (1·25 g.) was boiled for a few minutes; all then dissolved. When the clear solution was made weakly acid to Congo-paper, the product was precipitated as a filmy crystalline substance which easily gelated. This was washed with water in a centrifuge and dried on a porous plate and finally in a vacuum. Yield, 2 g.; m. p. 187° (decomp.) (Found: N, 9·3; As, 16·4. $C_{14}H_{20}O_5N_3S_2As$ requires N, 9·3; As, 16·7%).

Di(o-*carboxyphenyl*) *acetanilide*-p-*thioarsinite*. The arsenoxide (3·38 g.) was added to a solution of *o*-thiolbenzoic acid (4·62 g.) in water (50 c.c.) containing 2·52 g. of sodium hydrogen carbonate, and after the usual procedure 6·85 g. of the product were obtained. It is sparingly soluble in the common organic solvents, but can be crystallised from glacial acetic acid in microscopic plates, m. p. 225° (Found: As, 14·1, 14·3. $C_{22}H_{18}O_5NS_2As$ requires As, 14·5%).

Di(m-*carboxyphenyl*) *acetanilide*-p-*thioarsinite* was prepared in exactly the same way as the *o*-derivative described above. Yield, 92% of the theoretical. It separates from glacial acetic acid in microcrystalline granules, m. p. 219° (Found: As, 14·7. $C_{22}H_{18}O_5NS_2As$ requires As, 14·5%).

Di(glutathionyl) acetanilide-p-*thioarsinite.* Glutathione was prepared from yeast by Pirie's method (*Biochem. J.*, 1930, **24**, 51) and purified according to the procedure of Hopkins (*J. Biol. Chem.*, 1929, **84**, 269). It had m. p. 187—190°, and $[\alpha]^{20°}_{5461}$ — 19·01° in water. The glutathione (0·92 g.) was dissolved in water (10 c.c.). To this was added the parent arsenoxide (0·37 g., 10% excess). The containing vessel was filled with hydrogen and kept at room temperature for 2 days, during which time most of the arsenoxide passed into solution. The weight of residual arsenoxide collected on a filter indicated complete reaction. The clear solution was evaporated in a vacuum over sulphuric acid and the resulting clear gum was ground with successive portions of alcohol until a white powder was obtained. This was extremely hygroscopic and attempts to obtain crystals failed. The product decomposed at 115° (efferv.) (Found : N, 11·4; As, 8·5. $C_{28}H_{40}O_{13}N_7S_2As$ requires N, 11·9; As, 9·1%). Apart from its high solubility in water, this compound possesses all the characteristic properties of the thioarsinites.

Di(carbamylmethyl) acetanilide-p-*thioarsinite.* The parent arsenoxide (1·2 g.) was added to a solution of α-thiolacetamide (1 g.) in water (50 c.c.) and boiled for a few minutes. The solution was filtered clear. The *thioarsinite* separated on cooling (1·6 g.). It crystallises from water in long flat needles, m. p. 120° (Found : loss at 100°, 4·5. $C_{12}H_{16}O_3N_3S_2As,H_2O$ requires H_2O, 4·4%. Found for anhydrous material : N, 10·5. $C_{12}H_{16}O_3N_3S_2As$ requires N, 10·8%). The behaviour of this substance in alkali and bicarbonate is the same as that of the benzamide analogue.

Dithioarsinites derived from Benzamide-p-*arsenoxide.*—The thioarsinites in this section were prepared under exactly the same conditions as those described above for their acetanilide analogues.

Oxidation of Di(carboxymethyl) Benzamide-p-*thioarsinite.*—(a) *With one molecular proportion of oxygen.* A solution of this thioarsinite (0·754 g.; Gough and King, *loc. cit.*) in half-saturated sodium hydrogen carbonate solution was treated with 4·2 c.c. of a standard hydrogen peroxide solution (equivalent to 0·07 g. of hydrogen peroxide) at room temperature. The hydrogen peroxide was instantly consumed (vanadic acid test), but the nitroprusside reaction of a test portion to which caustic alkali was added was still positive. The solution was made acid to Congo-paper, and extracted with ether. Evaporation of the ether gave disulphidoacetic acid, m. p. 106°, and the solid insoluble in both layers was identified as the original thioarsinite. On concentration of the aqueous solution benzamide-*p*-arsonic acid crystallised in plates.

(b) *With two molecular proportions of oxygen.* The experiment was carried out as above, twice as much hydrogen peroxide being used. The nitroprusside reaction was negative at the end. The ether extract of the acidified solution gave 0·3 g. of disulphidoacetic acid, m. p. 106°, and benzamide-*p*-arsonic acid (0·35 g.) was obtained from the concentrated acid solution.

Di(β-*carboxyethyl*) *benzamide*-p-*thioarsinite* was obtained in 95% yield, and crystallised from 23 parts of water in fern-like leaflets, m. p. 160° (Found: As, 18·9; N, 3·4. $C_{13}H_{16}O_5NS_2As$ requires As, 18·5; N, 3·5%).

Di(β-*carboxy*-β-*aminoethyl*) *benzamide*-p-*thioarsinite*. This separated from the weakly acid reaction liquid as a heavy microcrystalline powder in 93% yield. By reprecipitation from very dilute solution it was obtained in long needles, which decomposed at 240° (Found: As, 17·2; N, 9·3. $C_{13}H_{18}O_5N_3S_2As$ requires As, 17·2; N, 9·6%). It is sparingly soluble in organic solvents.

Di(o-*carboxyphenyl*) *benzamide*-p-*thioarsinite* was obtained in 90% yield. It is sparingly soluble, and crystallises from a large volume of glacial acetic acid in small leaflets, m. p. 247° (efferv.) (Found: N, 2·5; As, 14·5. $C_{21}H_{16}O_5NS_2As$ requires N, 2·8; As, 14·9%).

Di(m-*carboxyphenyl*) *benzamide*-p-*thioarsinite* was obtained in almost theoretical yield. It separates from glacial acetic acid as a microcrystalline powder, m. p. 285° after softening at 270° (Found: As, 14·8. $C_{21}H_{16}O_5NS_2As$ requires As, 14·9%).

Di(*glutathionyl*) *benzamide*-p-*thioarsinite*, m. p. 130° (decomp.), was prepared in almost theoretical yield (Found: N, 11·6, 11·8, 12·4; As, 9·2. $C_{27}H_{38}O_{13}N_7S_2As$ requires N, 12·1; As, 9·3%). Some specimens of this substance give no nitroprusside reaction in bicarbonate solution, others a weak reaction.

Di(*carbamylmethyl*) *benzamide*-p-*thioarsinite* was obtained in 95% yield. It crystallises from water in small needles, m. p. 193° (Found: N, 10·95. $C_{11}H_{14}O_3N_3S_2As$ requires N, 11·2%). It is insoluble in sodium hydrogen carbonate solution, the suspension giving no nitroprusside reaction; in cold 0·1*N*-sodium hydroxide, however, it is completely soluble and gives an intense nitroprusside reaction. The solution, on saturation with carbon dioxide, deposits needles, the m. p. of which (193°) is not depressed by admixture with the original thioarsinite.

Di(β-*carboxy*-β-*aminoethyl*) *benz*-p-*thioarsinite*. (*a*) A solution of cystine (0·48 g.) in 0·4*N*-sodium hydroxide (10 c.c.) was added to a solution of 4 : 4′-arsenobenzoic acid (0·39 g.) in 0·4*N*-sodium hydroxide (5 c.c.). A further 10 c.c. of *N*-sodium hydroxide were added and the mixture was heated on the water-bath for 30 minutes. The yellow colour was gradually discharged. After filtration the

solution was made weakly acid to Congo-paper; woolly needles then separated (0·6 g.). The substance was purified by reprecipitation from a warm, weakly acid, dilute solution and obtained in fine needles, decomp. 245° (Found: N, 6·5. $C_{13}H_{17}O_6N_2S_2As$ requires N, 6·4%).

(*b*) The condensation of *p*-benzarsenoxide with cysteine was carried out as described above for other cysteine derivatives. An excellent yield of the required product was obtained and this was found to be identical with the substance described in (*a*). It is soluble in sodium hydrogen carbonate solution, but the nitroprusside reaction is absent.

Attempts to prepare Thioarsinites from a 2-Thiolglyoxaline.—Neither acetanilide-*p*-arsenoxide nor benzamide-*p*-arsenoxide would dissolve when boiled with sodium hydrogen carbonate solutions of 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylic acid (Balaban and King, J., 1927, 1865) and in each case the starting materials were recovered unchanged. The ability of this thiol compound to undergo a simpler condensation was shown with monochloroacetic acid. The thiolglyoxaline derivative (3·16 g.) was neutralised with sodium hydrogen carbonate in water (25 c.c.). A solution of monochloroacetic acid (1·9 g.) in water (25 c.c.) containing 3·36 g. of sodium hydrogen carbonate was added, and the mixture boiled for 45 minutes. The solution was made weakly acid to Congo-paper and cooled. The product crystallised from 13 parts of water, giving *2-carboxymethylthiol-4*(or 5)-*methylglyoxaline-5*(or 4)-*carboxylic acid* in rectangular plates, decomp. 188° (Found: loss at 95°, 7·4. $C_7H_8O_4N_2S,H_2O$ requires loss, 7·7%. Found for dried material: N, 12·6. $C_7H_8O_4N_2S$ requires N, 12·9%).

Cyclic Dithioarsinites.—cyclo*Ethylene acetanilide*-p-*thioarsinite* (III, X = NHAc). Dithiolethane (1·0 g.) was added to a suspension of acetanilide-*p*-arsenoxide (2·25 g.) in alcohol (50 c.c.), and the mixture boiled. After 10 minutes the solution was clear, and from it a felt of needles (2·5 g.) separated on cooling. A further crop of 0·3 g. was obtained on dilution of the warm mother-liquor with water. The product is soluble in 3 volumes of hot glacial acetic acid, and separates therefrom in pointed leaflets, m. p. 155°. From benzene solution large lustrous leaflets are obtained on the addition of light petroleum (Found: As, 24·6. $C_{10}H_{12}ONS_2As$ requires As, 24·9%). This substance is readily soluble in most organic solvents, and slightly so in ether. It is decomposed by both acid and alkali as indicated by the development of the characteristic odour of the mercaptan.

cyclo*Ethylene benz*-p-*thioarsinite* (III, X = CO_2H) was prepared in a similar manner to the last substance, by employing *p*-benz-

arsenoxide. It crystallises from alcohol in long leaflets, m. p. 223—224° (Found : As, 26·0. $C_9H_9O_2S_2As$ requires As, 26·0%). It resembles the acetanilide analogue in its properties.

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