The chemotherapy of antimony. Comparison of the antimonyltartrates with the organic compounds of antimony / by Robert George Fargher and William Herbert Gray.

No. 192

THE CHEMOTHERAPY OF ANTIMONY

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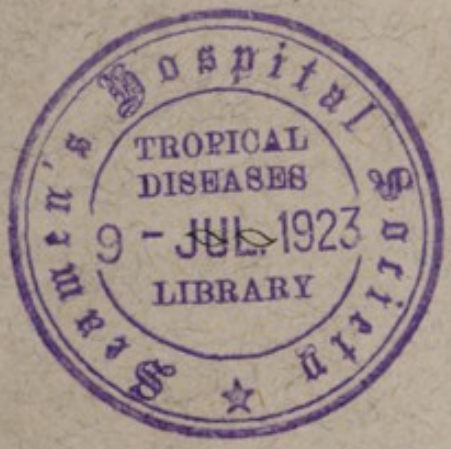
(From the Journal of Pharmacology and Experimental Therapeutics, 1921, *Vol.* 18)



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Reprinted from THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. xviii, No. 5, December, 1921

THE CHEMOTHERAPY OF ANTIMONY. COMPARISON OF THE ANTIMONYL TARTRATES WITH THE ORGANIC COMPOUNDS OF ANTIMONY

ROBERT GEORGE FARGHER AND WILLIAM HERBERT GRAY

From the Wellcome Chemical Research Laboratories, London

Received for publication June 14, 1921

Potassium antimonyl tartrate was first described in 1631 by Adrian de Mynsicht. Its preparation may have been suggested by a powder invented by Dudley, Earl of Warwick, and composed of scammony, antimony sulphide, and potassium tartrate ground together, of which an account was given in a treatise by Cornachinus called "Methodus in Pulverem," published in Italy in 1620; or by the practice of drinking wine which had been allowed to stand in an antimony goblet.

Early in the nineteenth century it was analyzed by a number of investigators with contradictory results (1) and was assumed to have the composition $C_4H_4O_7SbK,H_2O$ (in modern notation) until the careful work of Dumas and Piria (2) showed that it was in reality $C_4H_4O_7SbK,\frac{1}{2}H_2O$. The same observers showed that the corresponding sodium salt was $C_4H_4O_7SbNa,\frac{1}{2}H_2O$ and the ammonium salt $C_4H_8O_7NSb,\frac{1}{2}H_2O$. Another hydrate of the latter with $2\frac{1}{2}H_2O$ was afterwards discovered by Berlin (3) whilst the present writers have obtained a third hydrate containing $1\frac{1}{2}H_2O$. Dumas and Piria (4) considered the barium salt to be $C_8H_8O_{14}Sb_2Ba,2\frac{1}{2}H_2O$, although in this case their figures do not seem to have been so reliable. Careful analysis of a number of preparations now shows that three molecules of water are present.

The lithium salt was described by Buchner (5) as a transparent gelatinous mass from which small crystals separated very slowly. It was found by Plimmer and Thomson (6) to have a strong trypanocidal action, but it is not clear that a crystalline speci-

men was used. The authors have obtained it in large well formed crystals.

The aniline and quinine salts appear to have been first described by Clarke (7). The former was given the composition $C_4H_4O_6.SbOH.C_6H_5NH_2$, but Yvon (8) has since described a hydrated form, $C_4H_5O_6(SbO)C_6H_7N,H_2O$, which is efflorescent and consequently of variable antimony-content. On this account he recommends the adoption of the anhydrous salt in medicine. The quinine salt was obtained as an amorphous powder, but the present writers have succeeded in crystallising it.

Serious attempts to employ antimony in medicine have been attributed to Basil Valentine, under the influence of whose teaching it came to be looked upon as a universal panacea—so much so that towards the end of the seventeenth century there were more than a hundred preparations of the metalloid in more or less common use. During the last decade, however, antimony has assumed a new importance from the use of tartar emetic in certain tropical diseases of parasitic origin. The present position was recently outlined by one of us (9) and the conclusion drawn that results of sufficient importance had been obtained in the treatment of trypanosomiasis, bilharziasis, and leishmaniasis to necessitate search for more favourable means of presenting antimony. For some time prior to this, experiments had been in progress with the object of studying (a) the effect of variation of the basic radicle in the emetics, (b) the replacement of tartaric acid by other suitable acids, and (c) the question of the relative merits of the true organic compounds of antimony and the emetics.

So far, representative specimens of these three types have been prepared, and their toxicity determined by intravenous injection in mice. The toxicological work has been undertaken by Dr. J. Trevan, of the Wellcome Physiological Research Laboratories, and the authors are much indebted to him for his kindness in devoting so much time and care to this part of the investigation. Dr. Trevan proposes to examine the precise pharmacological action of the drugs.

Trials of all the members of this series on diseases experimentally induced in rats and other animals are also in progress. For purposes of discussion the results now published may conveniently be divided thus:

1. THE SALTS OF ANTIMONYL TARTARIC ACID

It was supposed that the antimony in tartar-emetic formed part of a basic radicle antimonyl (SbO) until Clarke and Stallo (10), in 1880, isolated ortho-antimonious acid, $Sb(OH)_3$, and submitted that the emetics were derived from this by replacing two of its hydroxyl groups by the divalent group $C_4H_4O_6$. Shortly afterwards Jungfleisch (11) suggested that the reactive groups in the tartaric acid were the alcoholic hydroxyl groups, esters which still retained an acidic function being formed.

This conception has been supported by the evidence of Guntz that the formation of antimonyl tartrates is, like ester-formation, endothermic (12); by that of Adam, who showed that addition of alkali to a tartar emetic solution caused immediate evolution of heat—evidence of the presence of a free carboxyl group—and subsequently a slow liberation of antimony oxide resembling saponification(13); and by the recent work of Blanchetière, who found that the velocity of solution of antimony trioxide in tartaric acid solutions, at first great, afterwards rapidly diminished, complete solution being attained only after an extended period (14).

The chemical results of the present investigation are generally in accord with this view, and it may be noted that the aniline and glyoxaline salts are sufficiently acid in reaction to require neutralization before injection, though when crystallization in presence of a second molecule of base was attempted the acid salt still separated.

The pharmacological results are interesting and somewhat unexpected, as it has been found possible to reduce considerably the toxicity per unit weight of antimony presented (see table on p. 358). The salts examined comprised those of:

a. The alkali metals: potassium, ammonium, sodium and lithium. These showed decreased toxicity and increased

solubility in water in the order named, thus confirming Rogers' contention (15) that the sodium salt is slightly, though definitely, less toxic than the potassium salt. The authors have been unable to confirm the statement of Plimmer and Thomson (6) that the lithium salt is more toxic than the sodium salt; in their experience it has a notably low toxicity, the amount of antimony in the minimum lethal dose being two and a half times as great as that of the potassium salt and nearly double that of the sodium salt (see table). Comparative measurements of surface tension and viscosity were also made, but these showed no parallelism to toxicity.

b. The cinchona alkaloids: quinine, quinidine, hydroquinine, cinchonine, cinchonidine and quinotoxine. Of these quinine has proved much the least toxic, the toxicity per unit weight of antimony presented being only one-fifth that of tartar emetic. In this series no obvious connection exists between toxicity and solubility or other physical properties.

c. Aniline and p-*phenetidine.* Yvon (8) has claimed that the aniline salt showed low toxicity in hypodermic injection and considerable activity against trypanosomes. In our experience the aniline salt differs but little in toxicity from the potassium salt when compared over a period of eight days.

The less soluble *p*-phenetidine salt is, however, more favourable, the toxicity per unit weight of antimony presented being roughly one quarter that of tartar emetic.

d. Ethylenediamine and butylamine. Of these, the former is considerably more toxic than tartar emetic, whilst the latter is roughly one-third as toxic per unit weight of antimony presented.

e. Glyoxaline. This salt, like that of aniline, required neutralization before injection. It is roughly only one-half as toxic as tartar emetic per unit weight of antimony presented.

Generally, then, the conclusion may be drawn that by variation of the base in the antimonyl tartrates considerably decreased toxicity per unit weight of antimony presented may be attained, the most favourable salts being those of quinine and *p*-phenetidine. The differing behaviour of aqueous solutions of quinine and quinidine tartrates on boiling with antimony trioxide is

noteworthy, the quinine being transformed almost completely into the more toxic quinotoxine, whilst the stereoisomeride, quinidine, is unaffected.

2. REPLACEMENT OF TARTARIC ACID BY OTHER ACIDS

Thomson and Cushny (16) investigated the trypanocidal action of a number of combinations of antimony with other organic acids, including lactic, citric, malic, and mucic acids, and came to the conclusion that these offered no advantage over the antimonyl tartrates. Rowntree and Abel (33) tested the efficacy of sodium antimony-thioglycollate and the triamide of the corresponding acid in experimental trypanosomiasis and found them compare favourably with the other antimonials.

The results which the authors have so far obtained with other acids, show little pharmacological interest. Chemically they support strongly the accepted view of the constitution of the antimonyl tartrates, and in cases where combination has been effected more drastic treatment has been necessary, whilst the products compare unfavourably with the antimonyl tartrates from the standpoint of ready purification.

3. THE ORGANIC COMPOUNDS OF ANTIMONY

So long ago as 1911, the *m*-amino derivatives of triphenylstibine oxide and of diphenylstibinic acid, monophenylstibinic acid, and certain of their reduction products were found to have a slight trypanocidal action, but were very irritant when administered subcutaneously (17).

The use of the intravenous route marked an important advance and the extension of the Bart reaction to the aromatic antimonials (18) has stimulated interest as it provides ready means of obtaining many hitherto difficultly accessible monoarylstibinic acids.

Thus, a number of recent communications (19, 20, 21) deal with the action of the sodium salts of *p*-aminophenylstibinic acid and its derivatives in experimental spirochaete and trypanosome infections, whilst Schmidt (22) has studied a number of compounds of this type exhaustively and has presented some

of the conclusions we had reached already. The alkali salts of the arylstibinic acids were described by Hasenbäumer (23) as easily hydrolysable substances; the ammonium salts he assumed to be incapable of existence. Further examination by the Chemische Fabrik von Heyden (24) indicated that salts of the types R.SbO(OH)(OM) or $R.SbO(OM)_2$ reacted alkaline in aqueous solution and that the neutral salts contained less than one atomic proportion of the alkali metal to a molecular proportion of the stibinic acid. In the case of phenylstibinic acid Schmidt (22) has since obtained initial neutralization when the ratio Na: Sb is approximately 1:3, but on standing the solution acquires an acid reaction, and if this be progressively removed by the addition of further alkali final neutrality is only reached after about a day at ordinary temperature, when the ratio becomes approximately 1:1. He explains this by the assumption that the free acid has a condensed molecule formed from three simple molecules of $C_6H_5SbO_3H_2$, and is gradually depolymerised in solution by alkali.

The authors have determined sodium and antimony in the isolated neutral salts of phenylstibinic acid and its *m*-acetylamino-, *p*-acetylamino-, *p*-bromo-, and *p*-ethoxy-derivatives. In the acetylamino-compounds the atomic ratio Na: Sb is approximately 1:3 but in the others it is greater, reaching 1:1.27 in the case of sodium *p*-ethoxyphenylstibinate. Since the methods of preparation differed in the two groups, the former being precipitated by sodium chloride from a solution saturated with carbon dioxide, and the latter formed by dissolving the free acid in alkali, it appears that a partial depolymerisation has occurred in the latter case and a mixture of salts, containing more sodium than the salt of the polymerised acid, has been formed.

In no case have the salts been obtained in a crystalline condition, and the same is true of numerous parallel attempts with the potassium and lithium salts. Sodium *m*-acetylaminophenylstibinate is the least toxic—only one twelfth as toxic as tartar emetic per unit weight of antimony presented—whilst the corresponding *p*-derivative is one-tenth as toxic. The others are much less favourable.

It is of interest to record that Caronia (25) has found sodium *p*-acetylaminophenylstibinate effective in a limited number of cases of infantile leishmaniasis of the form encountered in the Mediterranean area, whilst Manson-Bahr (26) has recorded its utility in a single case of trypanosomiasis (t. gambiense). On the other hand Wallace tested its effect in a case of trypanosomiasis (32) and considered it less effective than tartar emetic, and Manson-Bahr found it of no value in bilharziasis or benign tertian malaria (loc. cit.).

4. ANALYTICAL PROCEDURE

a. The estimation of antimony in organic compounds. Until recently the only method described in the literature was that of Cahen and Morgan (27). Schmidt (22) has employed a second method, involving a modified Kjeldahl estimation. Both of these are cumbersome and tedious, so the present authors have employed a variant of the modified Lehmann method for the estimation of arsenic devised by one of them (28) which gives excellent results.

b. The estimation of sodium. This is sufficiently indicated in the experimental portion.

5. EXPERIMENTAL

Salts of antimonyl tartaric acid

Two well-known methods have been employed in the preparation of these. In the first, molecular proportions of tartaric acid and antimony trioxide are heated in boiling aqueous solution with a molecular proportion of a monacidic base or the equivalent quantity of a di- or polyacidic base. In the second, equivalent quantities of barium antimonyl tartrate and the sulphate of the base are allowed to interact in aqueous solution. Unless otherwise stated the first method has been used.

Barium antimonyl tartrate

This salt separates from water in hexagonal plates or flattened prisms containing 3 molecules of water of crystallisation, and not

$2\frac{1}{2}$ as stated by Dumas and Piria (4). Found, loss at 110° in three specimens, 7.0, 7.1, 7.0 per cent. $C_8H_8O_{14}Sb_2Ba, 3H_2O$ requires H_2O = 7.1 per cent.

Ammonium antimonyl tartrate

A third hydrate of this salt has been obtained, separating from water in large flattened prisms containing one and a half molecules of water of crystallisation. It is practically insoluble in alcohol, but readily so in water, 100 cc. of water at 18° dissolving 66 grams of the hydrate.

Found, loss at 110° = 7.8 per cent. $C_4H_8O_7NSb, 1\frac{1}{2}H_2O$ requires 8.2 per cent. In dried material, Sb = 40.0. $C_4H_8O_7NSb$ requires Sb = 39.7 per cent.

Lithium antimonyl tartrate

In consequence of the ready solubility of this salt, considerable difficulty was experienced in obtaining it in a crystalline condition. This was eventually accomplished by seeding the solution, concentrated to a syrup, with a fragment of the crystalline ammonium salt, and recrystallising the ill-defined solid mass which gradually formed from boiling dilute alcohol, when large glistening octahedra were obtained. These retained $2\frac{1}{2}$ molecules of water, which were given off on drying at 60°, or over sulphuric acid, in a vacuum.

Found, loss over H_2SO_4 in a vacuum = 13.5, 13.6 per cent, $C_4H_4O_7SbLi, 2\frac{1}{2}H_2O$ requires 13.4 per cent. In air dried material, C = 14.4; H = 2.35; $C_4H_4O_7SbLi, 2\frac{1}{2}H_2O$ requires C = 14.3; H = 2.7 per cent. In dried material, C = 16.7; H = 1.4; Sb = 40.9 per cent. $C_4H_4O_7SbLi$ requires C = 16.5; H = 1.4; Sb = 41.2 per cent. One hundred cubic centimeters of water at 18° dissolve 180 grams of the hydrated salt.

Ethylenediamine antimonyl tartrate

This salt separates from water as a serrated mass of flattened prisms which contain a molecule of water of crystallisation and dissolve somewhat sparingly in water or in alcohol. On heating it darkens about 280°, but remains unmelted at 300°. One hun-

dred cubic centimeters of water at 18° dissolve 4.0 grams of the hydrated salt.

Found, loss at 110° = 2.4. $C_{10}H_{18}O_{14}N_2Sb_2,H_2O$ requires 2.7 per cent. In dried material, C = 19.3; H = 3.0; N = 4.5; Sb = 38.3. $C_{10}H_{18}O_{14}N_2Sb_2$ requires C = 19.0; H = 2.9; N = 4.4; Sb = 38.1 per cent.

Butylamine antimonyl tartrate

When prepared by the "boiling" method, this salt did not crystallise from water or alcohol, in both of which it dissolved readily, but separated from boiling benzene containing a little alcohol in hexagonal prisms containing three-quarters of a molecule of benzene.

Found, loss at 60° in a vacuum = 12.8. $C_8H_{16}O_7NSb$, $\frac{3}{4}C_6H_6$ requires 13.5 per cent. The volatile solvent was identified as benzene. The product was, however, unsatisfactory, as it persistently retained the last traces of benzene, so the alternative method of preparation was resorted to. On concentrating the aqueous solution obtained in this way to a syrup and keeping it for some time in a cold place, a radiating mass of elongated flattened prisms separated. These were recrystallised from alcohol and formed large rectangular plates containing two molecules of water, lost on drying over sulphuric acid in a vacuum, but regained rapidly on exposure to the air, the dried material being deliquescent. The hydrated substance melted at 40° (corr.), the anhydrous at 155° (corr.). 100 cc.of water at 18° dissolved 238 grams of the hydrated salt.

Found, loss over H_2SO_4 in a vacuum = 9.0. $C_8H_{16}O_7NSb$, $2H_2O$ requires 9.1 per cent. In dried material, C = 27.1; H = 4.1; Sb = 33.3. $C_8H_{16}O_7NSb$ requires C = 26.8; H = 4.5; Sb = 33.5 per cent.

Glyoxaline antimonyl tartrate

This salt separated from concentrated aqueous solution in well-defined flattened prisms containing 2 molecules of water of crystallisation. These dissolved very sparingly in alcohol and the other usual organic solvents, and, on heating, darkened but

did not melt at 300°. 100 cc. of water at 18° dissolved 46.7 grams of the hydrated salt.

Found, loss at 110° = 8.8. $C_7H_9O_7N_2Sb.2H_2O$ requires 9.0 per cent. In dried material, C = 24.1; H = 2.5; Sb = 34.1. $C_7H_9O_7N_2Sb$ requires C = 23.8; H = 2.6; Sb = 34.0 per cent. The salt was found to be somewhat acid in reaction and therefore required neutralization before injection.

Aniline antimonyl tartrate

This salt crystallised from water or from alcohol in well-formed elongated rhombic prisms containing one molecule of water of crystallisation (compare Yvon, (8)), and melted at 162° (corr). Like the previous salt it required neutralisation before injection.

One hundred cubic centimeters of water at 18° dissolve 18.5 grams of the hydrated salt.

Found, loss at 110° = 4.4, $C_{10}H_{12}O_7NSb,H_2O$ requires 4.5 per cent. In dried material, C = 31.5; H = 3.2; Sb = 31.5. $C_{10}H_{11}O_7NSb$ requires C = 31.7; H = 3.2; Sb = 31.8 per cent.

p-*Phenetidine antimonyl tartrate*

This salt separated rapidly from water, in which it is only sparingly soluble, as a felted mass of needles. It dissolved more readily in hot alcohol, from which it formed masses of needles containing one molecule of water of crystallisation. The hydrated salt melts and effervesces at 148°, and the anhydrous salt at 245° (corr.). 100 cc. of water at 18° dissolve 8.5 grams of the hydrated salt.

Found, loss at 100° in a vacuum = 4.7. $C_{12}H_{16}O_8NSb,H_2O$ requires 4.1 per cent. In dried material, C = 34.4; H = 3.9; Sb = 28.1. $C_{12}H_{16}O_8NSb$ requires C = 34.1; H = 3.8; Sb = 28.4 per cent.

Quinine antimonyl tartrate

This was originally prepared by Clarke (7) who did not, however, obtain it in a crystalline condition. Employing the same method—that of double decomposition between quinine sulphate and barium antimonyl tartrate—it has now been ob-

tained in slender glistening needles containing a molecule of water of crystallisation. One hundred cubic centimeters of water at 18° dissolve 1.01 grams of the hydrated salt, although solution was slow, and agitation for several days was found to be necessary.

Found, loss at 110°, 3.3, $C_{24}H_{29}O_9N_2Sb,H_2O$ requires 2.8 per cent. In dried material, C = 47.2; H = 4.8; Sb = 19.6. $C_{24}H_{29}O_9N_2Sb$ requires C = 47.4; H = 4.8; Sb = 19.7 per cent.

When quinine tartrate was boiled in aqueous solution with antimony trioxide, an amorphous yellow product was obtained. This dissolved much more readily in water than quinine antimonyl tartrate and did not crystallise. Its colour, increased toxicity, and the well known methods of converting quinine into quinotoxine indicate that it is probably quinotoxine antimonyl tartrate. The solution resulting from the interaction on concentration first deposited a small crop of crystals which proved to be quinine tartrate. On further evaporation, an oil separated which was obtained solid by boiling with a little alcohol but resisted all attempts at crystallisation.

Found, loss at 60° in a vacuum = 4.2. $C_{24}H_{29}O_9N_2Sb$, $1\frac{1}{2}H_2O$ requires 4.2 per cent. In dried material Sb = 20.5. $C_{24}H_{29}O_9N_2Sb$, requires Sb = 19.7 per cent.

Quinidine antimonyl tartrate

Unlike quinine, quinidine shows no tendency to transformation, and the same product is obtained from both processes. The salt separates from much water in slender glistening needles containing 4 molecules of water of crystallisation. One hundred cubic centimeters of water at 18° dissolve 0.31 gram of the hydrated salt. Compare Hesse (34).

Found, loss at 110° = 10.0. $C_{24}H_{29}O_9N_2Sb,4H_2O$ requires 10.5 per cent. In dried substance, C = 47.1; H = 4.9; Sb = 19.6. $C_{24}H_{29}O_9N_2Sb$ requires C = 47.3; H = 4.8; Sb 19.7 = per cent.

Cinchonine antimonyl tartrate

Two hydrates of this salt have been described by Traube, the one $(C_{23}H_{27}O_8N_2Sb)_2,3H_2O$, crystallising in monoclinic hemi-

morphs (29), the other $(C_{23}H_{27}O_8N_2Sb)_2,5H_2O$, in the hexagonal system (30). The authors have obtained a third, $(C_{23}H_{27}O_8N_2Sb)_2$, H_2O, crystallising readily from water in well-formed rectangular plates. 100 cc. of water at 18° dissolve 2.96 grams of the hydrated salt.

Found, loss at 60° in a vacuum = 1.3 per cent. $(C_{23}H_{27}O_8N_2Sb)_2$, H_2O requires 1.5. In dried material, C = 47.4; H = 4.7; Sb = 20.9. $C_{23}H_{27}O_8N_2Sb$ requires C = 47.6; H = 4.7; Sb = 20.7 per cent.

Cinchonidine antimonyl tartrate

Cinchonidine sulphate, found by analysis to be $(C_{19}H_{22}ON_2)_2$, $H_2SO_4,6H_2O$, was used in this experiment and converted into the antimonyl tartrate by double decomposition with barium antimonyl tartrate. The aqueous solution so obtained was concentrated to low bulk, treated with alcohol until the resulting oil redissolved, and set aside for several days in a vacuum over sulphuric acid, when large rectangular plates gradually formed. These proved to have the composition $(C_{23}H_{27}O_8N_2Sb)_2$, $5H_2O$, and melted at 192° (corr.). One hundred cubic centimeters of water at 18° dissolve 1.37 grams of the hydrated salt.

Found, loss at 60° in a vacuum = 6.7. $(C_{23}H_{27}O_8N_2Sb)_2,5H_2O$ requires 7.1 per cent. In dried material, C = 47.2; H = 4.8; Sb = 20.6, 20.9 per cent. $C_{23}H_{27}O_8N_2Sb$ requires C = 47.6; H = 4.7; Sb = 20.7 per cent.

Hydroquinine antimonyl tartrate

Hydroquinine sulphate, which proved to have the composition $(C_{20}H_{26}O_2N_2)_2H_2SO_4,6H_2O$, was converted into the antimonyl tartrate and the product isolated as in the case of the cinchonidine salt. The air dried material retained 5 molecules of water of crystallisation, and melted at 201° (corr). 100 cc. of water at 18° dissolve 2.24 grams.

Found, loss at 60° in a vacuum = 12.8. $C_{24}H_{31}O_9SbN_2,5H_2O$ requires 12.8 per cent. In dried material, C = 47.3; H = 4.8; Sb = 19.4. $C_{24}H_{31}O_9N_2Sb$ requires C = 47.1; H = 5.1; Sb = 19.6 per cent.

The organic compounds of antimony

These compounds were all prepared by interaction of diazonium salts with sodium antimonite. The mechanism of the reaction has been discussed recently by Schmidt (22); details of preparation of such substances as have been already described are therefore omitted.

Neutral sodium salt of m-acetylaminophenylstibinic acid

This was obtained as a pale pink amorphous powder which gave clear neutral solutions in water and alcohol. It was dried at 60° in a vacuum for analysis.

The figures obtained correspond to the formula

$$(C_2H_3O, NHC_6H_4\ SbO)_3,O_2\ (OH)(ONa),$$

so that the substance isolated under these conditions appears to be the sodium salt of a polymerised acid formed from three molecules of the acid $C_2H_3O,NHC_6H_4\ SbO_3H_2$ the existence of which has been indicated by Schmidt (22).

Found, Na = 2.65; Sb = 40.49 per cent., atomic ratio Na : Sb = 1: 2.9. $C_{24}H_{25}O_{10}N_3Sb_3Na$ requires Na = 2.56; Sb = 40.21 per cent; Na : Sb = 1 : 3.

In this and other cases it was found necessary to correct the results for the presence of admixed sodium chloride, which persisted after repeated extraction with anhydrous methyl alcohol.

Neutral sodium salt of p-*acetylaminophenylstibinic acid*

This was prepared from *p*-aminoacetanilide (18,24), and formed a faintly coloured granular powder, readily soluble in water or in methyl alcohol, which differed but little in toxicity from salvarsan. Figures comparable with those for the antimonyl tartrates are given in the table.

It was dried at 60° in a vacuum for analysis and was found to contain nearly three atomic proportions of antimony to one of sodium.

Found, Na = 2.81; Sb = 40.82 per cent; atomic ratio Na: Sb = 1 : 2.8. $C_{24}H_{25}O_{10}N_3Sb_3Na$ requires Na = 2.56; Sb = 40.21 per cent; ratio Na : Sb = 1 : 3.

Neutral sodium salt of p-*bromophenylstibinic acid*

A solution of 8.6 grams of *p*-bromoaniline in 180 cc. of water containing 12.2 cc. of hydrochloric acid (D = 1.16) was diazotised at 0° with 3.5 grams of sodium nitrite, and added to a cooled mixture of 7.2 grams of antimony trioxide dissolved in 35 cc. of concentrated hydrochloric acid and mixed with 40 grams of sodium hydroxide in 100 cc. of water containing crushed ice. No evolution of nitrogen occurred until the temperature had risen to 11°. The mixture was then further diluted with water and stirred mechanically for several hours, the temperature being kept between 11° and 15°C. The filtrate from the reaction product was decolorised with charcoal, made nearly neutral to litmus with dilute sulphuric acid, filtered from a small quantity of dark solid, and since a preliminary experiment had shown that the acid was precipitated at this stage by carbon dioxide, dilute acid was added until the reaction was just neutral to congo red. The pale buff precipitate was washed very thoroughly and ground with sufficient dilute sodium hydroxide to produce a neutral solution, this solution treated with charcoal, filtered and evaporated to dryness. The residue was extracted with cold dry methyl alcohol, and the clear filtrate evaporated to dryness in a vacuum. The product so obtained was readily soluble in water and methyl alcohol to a clear solution, neutral in reaction.

Here the ratio of sodium to antimony was found to be higher than in the previous cases. Partial depolymerisation and consequent formation of a mixture of sodium salts had therefore occurred during the solution of the *p*-bromophenylstibinic acid in alkali.

Found, Na = 4.89; Sb = 34.71; atomic ratio Na : Sb = 1: 1.35. A mixture of 36.8 per cent of $(C_6H_4BrSbO)_3O_2(OH)(ONa)$ and 63.2 per cent of $C_6H_4BrSbO(OH)(ONa)$ requires Na = 5.04; Sb = 35.51 per cent, and ratio Na : Sb = 1 : 1.35.

Neutral sodium salt of p-*ethoxyphenylstibinic acid*

A solution of 13.7 grams of *p*-phenetidine in 250 cc. of water containing 24.5 cc. of hydrochloric acid (D = 1.16) was diazo-

tised at 0° with 7 grams of sodium nitrite. The resulting solution was added to a cooled mixture of 14.4 grams of antimony trioxide dissolved in 70 cc. of hydrochloric acid (D = 1.16) and 80 grams of sodium hydroxide in 450 cc. of water containing crushed ice. The evolution of nitrogen was slow. Six hundred cubic centimeters of water were then added and the mixture stirred mechanically for several hours. A considerable proportion of tar formed. This was removed and the filtrate decolorised with charcoal, almost neutralised to litmus with dilute sulphuric acid and saturated with carbon dioxide. The white solid which separated was found to be insoluble in water but soluble in dilute alkalis, dilute mineral acids reprecipitating it in a gelatinous form. It was dissolved to a neutral solution by careful addition of normal sodium hydroxide, evaporated to dryness under diminished pressure and the residue extracted with dry methyl alcohol. The extract was evaporated and the residue again dissolved in water, a slight turbidity removed by filtration, and the solution obtained finally evaporated under diminished pressure and dried. The yield was only small—1.25 grams of a pale yellow solid. The filtrate remaining after saturation with carbon dioxide only yielded a trace of solid when saturated with sodium chloride.

Found, Na = 5.91; Sb = 39.37 per cent; atomic ratio Na: Sb = 1: 1.27. A mixture of 29.75 per cent of $(C_2H_5O.C_6H_4.SbO)_3O_2(OH)(ONa)$ and 70.25 per cent of $C_2H_5O.C_6H_4SbO(OH)(ONa)$ requires Na = 5.95; Sb = 39.43 per cent; Na : Sb = 1 : 1.27.

Neutral sodium salt of phenylstibinic acid

The corresponding acid was prepared substantially by the method described by Schmidt (22), and converted into the sodium salt by solution in sufficient sodium hydroxide to form a neutral solution and evaporation to dryness. The ratio of sodium to antimony showed that there also some depolymerisation had taken place.

Found, atomic ratio Na : Sb = 1 : 2.06. A mixture of 75.35 per cent of $(C_6H_5SbO)_3O_2(OH)(ONa)$ and 24.65 per cent of $C_6H_5SbO(OH)(ONa)$ requires Na : Sb = 1 : 2.06.

6. METHODS OF ANALYSIS

a. The estimation of antimony in organic compounds

Direct titration with standard iodine can be employed only in the case of compounds of trivalent antimony in which the remainder of the molecule does not absorb iodine, such as certain of the antimonyl tartrates. Many compounds require, however, preliminary destruction of the organic portion and to effect this the method of Cahen and Morgan (27) was first used. The results were somewhat inconsistent and the method is necessarily somewhat tedious so a variant of the modified Lehmann method employed by one of us in the case of arsenic (28) has been adopted, with success. With care quite concordant results can be obtained and these in the case of tartar emetic are in close agreement with the direct iodine titration. The details are as follows:

0.2 gram of the finely powdered substance is intimately mixed in a dry 300 cc. flask with one gram of finely powdered potassium permanganate, and 10 cc. of 50 per cent sulphuric acid are added quickly whilst shaking the flask. A second gram of permanganate is then introduced, followed by 10 cc. of concentrated sulphuric acid in small portions.

After the mixture has been allowed to stand for a few minutes, 10 cc. of water are added and the mixture then boiled for half an hour under a simple reflux condenser formed by a wide tube with a bulb to rest on the neck of the flask. Hydrogen peroxide is then added in slight excess, followed by 30 cc. of water, and the solution again boiled until a drop of a dilute solution of potassium permanganate gives a faint permanent pink colour. This is discharged by a drop of a dilute solution of oxalic acid, the solution is cooled, diluted to 150 cc., 2.5 grams of potassium iodide added, the flask stoppered and allowed to stand for an hour before titration with sodium thiosulphate N/10. A yellow colour often persists after all the iodine has disappeared, so that starch must be used as indicator. A control should be carried out simultaneously with the antimony compound omitted and the result corrected accordingly. The following figures were

obtained with a sample of crystallised tartar emetic air-dried without losing the transparency of the crystals.

By permanganate method: Sb = 36.1; 36.2; 36.1 per cent. By iodine titration: 36.1; 36.3 per cent. $C_4H_4O_7SbK,\frac{1}{2}H_2O$ requires Sb = 36.17 per cent.

b. The estimation of sodium in the sodium salts

A weighed quantity of the substance under examination was dissolved in water and the solution acidified slightly with dilute sulphuric acid. After standing half an hour the gelatinous precipitate was filtered off, very thoroughly washed with water containing a little sulphuric acid, the filtrate re-filtered if necessary until quite water-bright, evaporated to dryness and heated to drive off the excess of sulphuric acid. The residue contained traces of insoluble material, and to remove this it was extracted with boiling water and the extract filtered. Barium chloride was then added and the sulphate estimated in the usual way.

7. TOXICITY DETERMINATIONS

These measurements, as already stated, were made by Dr. J. Trevan of the Wellcome Physiological Research Laboratories. The substances were dissolved in normal saline, sterilised[1] and injected into the tail-vein of mice. The mice were then kept under observation for at least eight days. The figures given under the heading M.L.D. in the table (below) represent the dose in grams per kilo of body weight necessary to cause death in eight days. The principal chemical and physical data relating to the substances are also shown.

[1] On one or two occasions the formation of small quantities of a crystalline substance corresponding in appearance and antimony content with sodium pyroantimonate, $Na_2H_2Sb_2O_7,6H_2O$, has been observed during the sterilization of sodium p-acetylaminophenylstibinate in normal saline solution. This is apparently inhibited by the addition of a small quantity of sodium hydroxide solution. The observation is of interest in view of the experiments of E. Schmitz (31) on the stability of partially neutralized arylarsinic acids, but the comparatively small quantity of material available has so far prevented further examination of the phenomenon.

NO.	NAME	FORMULA	PERCENTAGE ANTIMONY	100 PARTS WATER DISSOLVE AT 18°	VISCOSITY OF 7.5 PER CENT SOLUTION AT 25°	SURFACE TENSION AT 20° DYNES/CM.	M. L. D. GRAMS PER KILO	WEIGHT OF ANTIMONY IN M. L. D. MILLIGRAMS	NO.
1	Potassium antimonyl tartrate	$C_4H_4O_7SbK,\frac{1}{2}H_2O$	36.17	7.2	1.137	73.31	0.016	5.7	1
2	Ammonium antimonyl tartrate	$C_4H_8O_7NSb,1\frac{1}{2}H_2O$	36.51	66.0	1.131	72.4	0.02	7.4	2
3	Sodium antimonyl tartrate	$C_4H_4O_7SbNa,\frac{1}{2}H_2O$	38.01	91.0	1.177	72.74	0.025	9.5	3
4	Lithium antimonyl tartrate	$C_4H_4O_7SbLi.2\frac{1}{2}H_2O$	35.75	180.0	1.134	71.5	0.04	14.3	4
5	Barium antimonyl tartrate	$C_8H_8O_{14}Sb_2Ba,3H_2O$	31.63	0.49			0.01	3.2	5
6	Quinine antimonyl tartrate	$C_{24}H_{29}O_9N_2Sb,H_2O$	19.13	0.10			0.15	31.0	6
7	Quinidine antimonyl tartrate	$C_{24}H_{29}O_9N_2Sb,4H_2O$	17.61	0.31			0.02	3.5	7
8	Quinotoxin antimonyl tartrate	$C_{24}H_{29}O_9N_2Sb,1\frac{1}{2}H_2O$	18.86	25.0	1.223	60.19	0.023	4.0	8
9	Hydroquinine antimonyl tartrate	$C_{24}H_{31}O_9N_2Sb,5H_2O$	17.11	2.24			0.03	5.1	9
10	Cinchonine antimonyl tartrate	$C_{23}H_{27}O_8N_2Sb,\frac{1}{2}H_2O$	20.46	2.96			0.05	10.2	10
11	Cinchonidine antimonyl tartrate	$C_{23}H_{27}O_8N_2Sb,2\frac{1}{2}H_2O$	19.24	1.37			0.05	9.6	11
12	Ethylenediamine antimonyl tartrate	$C_{10}H_{18}O_{14}N_2Sb_2,H_2O$	37.06	4.0	(3%) 1.054	(3%) 72.51	0.005	1.85	12
13	Butylamine antimonyl tartrate	$C_8H_{16}O_7NSb,2H_2O$	30.48	238.0			0.045	15.0	13

14	Aniline antimonyl tartrate	$C_{10}H_{12}O_7NSb,H_2O$	30.33	18.5	1.163	71.09	0.02	6.0	14
15	*p*-Phenetidine antimonyl tartrate	$C_{12}H_{16}O_8NSb,H_2O$	27.29	8.5	1.191	60.29	0.08	21.6	15
16	Glyoxliane antimonyl tartrate	$C_7H_9O_7N_2Sb,2H_2O$	30.87	46.7	1.115	73.18	0.045	13.8	16
17	Sodium p-acetylaminophenylstibinate	$C_{24}H_{25}O_{10}N_3Sb_3Na$	40.12				0.133*	55.8	17
18	Sodium m-acetylaminophenylstibinate	$C_{24}H_{25}O_{10}N_3Sb_3Na$	40.12				0.174*	69.8	18
19	Sodium phenylstibinate	Partially polymerised mixture (see text)	46.56				0.072*	33.4	19
20	Sodium *p*-bromophenylstibinate	Partially polymerised mixture (see text)	34.71				0.019*	6.6	20

* Corrected for sodium chloride; compare page 353.

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