THE OGIU LABORATORY

Head : Prof. Dr. Kikuo Ogiu

Research on organo-arsenic compounds and that on technical improvements of preparing arsenobenzene (Saviol) were taken over by Dr. Kikuo Ogiu, Professor of pharmacology in the Faculty of Medicine and in October 1939, the Ogiu-Laboratory was established.

1. Chemistry and Pharmacology of organo-arsenic compounds

a) The migration of arsenic to the brain was recognised in the rabbit by the intravenous injection of 3-acetamino-4-hydroxyphenylarsonic acid, while scarcely any by the stomachic application.[1]

b) The toxic action of aromatic arsonic acids by different methods of administering was examined with mice.[2]

As material, 3-Nitro-4-hydroxy phenylarsonic acid I
3-Amino-4-hydroxy phenylarsonic acid II
3-Benzoylamino-4-hydroxy phenylarsonic acid III
3-Benzenesulfamino-4-hydroxy phenylarsonic acid IV
3-Carbamino-4-hydroxy phenylarsonic acid V
and 5-Acetamino-2-hydroxy phenylarsonic acid VI

were synthesized.

Each substance showed different toxicity according to the method of application, and it decreased in the following order.

Stomachal application : I>III>IV>VI>II> V
Subcutaneous injection : III>IV> I> V>VI>II
Intravenous injection : III> I>IV> V>II>VI

c) Comparison of the toxicity of ortho with that of para isomers of hydroxy phenylarsonic acids showed that the former was more toxic than the latter regardless of the methods of application.[3]

d) The toxic actions and the protozocidal actions of

3-Acetami-4-hydroxy phenylarsonic acid (VII)
3-Carbamino-4-hydroxy phenylarsonic acid (VIII)
3-Acetamino-4-acetoxy phenylarsonic acid (IX)
3-Acetamino-4-oxyacetic acid phenylarsonic acid (X)
and Phenmorphoron-3-arsonic acid (6) (XI)

which had been synthesized were examined by peroral, subcutaneous and intravenous application to mice.[4] The compounds (XI) and (VIII) showed intense toxicity, and (X) was less toxic than the others in each application method. The curative dose of each compound could not be established by intravenous injection. By peroral application, however, they showed small chemotherapeutic coefficients (C/T).

	Sp. recurrentis Duttoni	Trypanozoma gambiense
VII	1/9	2/9
VIII	2/7	1/7
IX	3/10	3/10
X	5/8	7/8
XI	2/5	1/5

e) Aerial oxydation of arsenobenzene.

The aeration of 3, 3′-diamino-4, 4′-dihydroxy arsenobenzene solution causes color change, turbidity, precipitation and increase in its toxity. The aerial oxidation product of arsenobenzene was studied by spectroscopic method.[5] The absorption band of arsenobenzene moves from 3100Å to 2920Å by aeration of 96 hours, while the absorption band of 3-amino-4-hydroxy phenylarsinoxide is observed at 2920Å. It is clear that oxidative rupture takes place in the arseno group, converting to arsinoxide. However, during the process of aeriation of arseno compound, the absorbed quantity of oxygen was about twofold of the value calculated from the titration of phenylarsinoxide, while 3-amino-4-hydroxy phenylarsin oxide could not be oxidized to corresponding arsonic acid by mere aeration. This means that the oxydation occurs not only in arseno binding but also in ortho amino-hydroxy group of arsenobenzene.

The variation of toxicity in the course of aeration differs according to the kind of amino group in arsenobenzene molecule.[6] Toxic action of 3, 3′-diamino-4, 4′-dihydroxy arsenobenzene fluctuated, and a relatively small increase was observed, while 3-amino-3′-aminosulfoxlate derivative (Neo-arsenobenzene) and 3, 3′-diaminosulfite derivative (Myo-arsenobenzene) were found to have extraordinary high toxicity and no decrease. Analogous difference was also observed between 3-amino-3′-glycyl- and 3, 3′-diacetamino-dihydroxy arsenobenzene.[7] Thus the contribution of the imino group to the toxification due to aerial oxidation is more drastic than that of the amino group. The insoluble part of oxidation product of arsenobenzene indicated that it is composed mainly of arseno compound, and its toxity[8] and spirochaetocidal action[9] increase according to the duration of aeriation. By further oxidation the soluble part of product, which contains arsin oxide, decreased its toxicity[8] and spirochaetocidal action.[9] It is therefore, conceivable that the arsenobenzene, of which the ortho amino-hydroxy group was only oxidized, meant higher toxicity and activity. Simultaneously high toxic 3-amino-4-hydroxy phenylarsin oxide was produced readily by oxidative rupture of the arseno group, and further aeration caused decrease of its toxicity due to the oxidation of the ortho amino-hydroxy group. Though the toxic substance of oxidized arseno benzene was not isolated, it seems certain that the formation of a kind of arseno compound other than phenylarsin oxide makes toxicity and activity increase.

The results of further research are described in the following articles:

f) An electro-cardiographic study of the action of arsenobenzene and its aerial oxidation product on the rabbit heart.[10]

g) Influence of some drugs on the respiration- and circulation-hindrance caused by the application of large amounts of arsenobenzene.[11]

h) Variation of toxicity[12] and activity[13] of arsenobenzene during their preservation.

i) Influence of arsenic compounds on enzymatic action.[14]

1) Chuichi Tomita and Tsuguyoshi Hojo: Folia Pharmacologica Japonica, **27**, 128 (1939).
2) Chuichi Tomita: *Ibid.*, **27**, 205 (1939).
3) Chuichi Tomite: *Ibid.*, **27**, 216 (1939).
4) Shigeru Matsuo: *Ibid.*, **38**, 72, 170, 184 (1943).
5) Koeki Kondo: *Ibid.*, **27**, 235 (1939).
6) Koeki Kondo *Ibid.*, **27**, 324 (1939); *Ibid.*, **28**, 1 (1940).
7) Koeki Kondo: *Ibid.*, **28**, 98 (1940).
8) Koyu To: *Ibid.*, **33**, 161 (1941).
9) Koyu To and Hitomi Akasawa: *Ibid.*, **33**, 320 (1941).
10) Toshio Akita and Koeki Kondo: *Ibid.*, **26**, 128 (1938).
11) Koeki Kondo and Sungwoo Kin: *Ibid.*, **28**, 109 (1940).
12) Masashichi Yoshioka, Susumu Otani and Kazuyuki Shimoi: Bull. Inst. Chem. Res., Kyoto Univ., **13**, 24 (1944).
13) Masashichi Yoshioka and Takashi Seki: *Ibid.*, **14**, 7 (1947).
14) Masashichi Yoshioka: Folia Pharmacologica Japonica, **31**, 289 (1941); Bull. Inst. Chem. Res. Kyoto Univ., **12**, 145, 159 (1941).
15) Susumu Otani: Folia Pharmacologica Japonica, **35**, 33 (1942).

2. Chemistry and Pharmacology of Aromatic Stibonic Acids and Antimony Compounds

Antimonials as therapeutic agents have a special value for several diseases, e. g., general infection called Kala-azar and an endemic caused by Schistosomum japonica for which arsenicals practically have no effect. Aromatic stibonic acids are a group of strictly organic antimonials, while organic antimonyl compounds are antimonials in which antimony, instead of being bound directly to a carbon atom, is separated from the latter by an atom of oxygen. Both kinds of antimonials are highly recommended for their protozocidal activities.

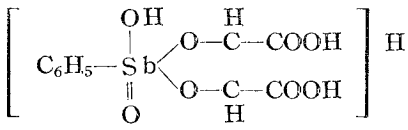
a) Among various salts of p-amino phenylstibonic acid, diethylammonium salt is fairly stable, while sodium salt is unstable, indicating the formation of insoluble matter during its preservation. However, a pharmacological study of diethylammonium p-amino phenylstibonate produced an unsatisfactory result in toxic action.[1]

b) Tartar emetic and corresponding sodium salt are also unstable in solution, giving turbidity. Sodium quinquivalent antimonyl gluconate was synthesized. This antimonyl compound is extraordinarily stable and low toxic, of which the distribution of antimony in animal bodies and histological change in various organs by repeated administration[2] and its influence on blood picture[3] were studied. The sodium quinquivalent antimonyl gluconate indicates special value for the chemotherapeutical effect not only on the Kala-azar of the rabbit[4] but also clinically on the human disease caused by Schistosomum japonica.[5]

c) Relating to instability, the preparation and the chemical properties of

aromatic stibonic acids were reexamined.[6] Phenylstibonic acid, prepared from ammonium phenyl pentachloro antimonate with alkali, contained different amounts of water, depending upon the condition of drying. Antimony content indicated the contamination of monomeric and dimeric stibonic acids. Both monomeric and dimeric stibonic acids had a monobasic character. The cryoscopic molecular weight determination of phenylstibonic acid using glacial acetic acid was unsuccesful owing to the dehydration and formation of acetic ester of monobasic dimer. From the behavior toward alkali the formula of phenylstibonic acid should be represented as $[C_6H_5Sb(OH)_5]H$, from which the intra-and inter-molecular dehydrations of the hydroxyl groups in complex anion produce various monobasic complex acids.

d) It was also found that the monomeric phenylstibonic acid could be converted to the corresponding antimonyl compound by treating with tartaric acid.[7] This phenyl quinquivalent antimonyl tartaric acid was a stable colorless crystal and its acidic monosodium salt was obtained, while acidic disodium salt could not be isolated. The formula of free acid may be represented as the following tribasic, where two hydroxyl groups, attached to quinquivalent antimonyl element of stibonic acid, are converted to antimonyl binding with α-carbon of α-hydroxy carboxylic acids.



e) Benzenediazonium-antimonytetrachloride complex salt, when treated with alkali, decomposed mainly into phenylstibonic acid (yield 37%) with the evolution of nitrogen in which hydrogen was acertained.[8] The complex salt, however, in organic solvent decomposed into chlorobenzene, benzene and a small amount of biphenyl, while in polar solvent with Cu_2Cl_2 or Cu as a catalyst it decomposed mainly into phenylstibonic tetrachloride, which was readily coverted to phenylstibonic acid. Methanol, acetone and acetic anhydride were apprpriate solvents, as the yield of phenylstibonic acid amounted to 60–70%. Concerning to the effect of substitution groups to the decomposition of complex salt, it was observed that the p-methyl group favoured the stability of the complex and yield of stibonic acid, while the p-ethoxy or p-nitro group depresses the stability and yield.

Thus it is clear that the decomposition of diazonium complex salt involves not only an ionic fission but also a non ionic one even in polar solvent.

1) Fumio Takenaka: Folia Pharmacologica Japonica **35**, 10 (1942).
2) Susumu Kanai: *Ibid.*, **42**, 161 (1947).
3) Susumu Kanai: *Ibid.*, **43**, 57 (1947).
4) Hiroshi Yamada and Takashi Seki: *Ibid.*, **41**, 279 (1944).
5) Hachiro Yoshida and Masao Morita: Yakugaku Kenkyu, **20**, 235 (1948).
6) Risaburo Nakai, Ryonosuke Toyoda and Hajime Tomono: Bull. Inst. Chem. Res., Kyoto Univ., **18**, 22 (1949).
7) Risaburo Nakai, Ryonosuke Toyoda and Hajime Tomono: *Ibid.*, **19**, 71 (1949).
8) Hajime Tomono: *Ibid.*, **21**, 41 (1950); **22**, 49 (1950); **23**, 45 (1950); **24**, 54 (1951).

3. Decarboxylation of Mercuric Salts of Organic Acids

In the decarboxylation reactions which occur in the thermal decomposition of metallic salts of organic acids, salts of mercury show salient points as compared

with that of other metals situated in the 1 and 2 groups of the periodic table.

When these pure or almost pure salts are heated as slowly as possible under 4 m.m. Hg pressure to their decomposition, behaviors of the salts, composed of alkalimetals of lower atomic weight, can be explained by means of establishing a connection with unstable intermediate compounds, whose existence can be reasonably expected in the fusing state of salt.

In case of alkali metals, these intermediate compounds are based on polarities of ions from two molecules of salts and, in case of alkaliearths, ions from one molecule.

The reaction products are metallic carbonates and ketones in above cases, and the same conception is applied to the reactions between the salt of formic acid and the same metallic salts of other fatty acids (the formation of aldehydes) and for metallic hydroxides instead of the salt of formic acid (the formation of hydrocarbon).[1]

To clarify the decomposition processes of mercuric salt, the above conception seems insufficient or rather unreasonable, and another type of reaction which proceeds by free radicals may play the role.

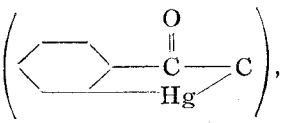
The condition of drying (to warm mercuric acetate on a water bath with passing air) caused its decomposition which was recognised by the appearance of the oxides of mercury in this material, and if this sample was heated, up to near 120°C, acetic acid was distilled out of this half-fused salt with gaseous products.[2]

Tendencies of this decomposition, e. g., the formation of metallic oxides, caused by air in the course of drying at a considerably lower temprature, were remarkable, when lead tetraacetate was worked out, and this is not only one of the striking differences between leadacetate and lead tetraacetate but also the points to be attended to the experiment with the compounds in which the element of component has large atomic weight and exhibits higher valency among its possible valencies.[3]

When the sample, without this change in drying, was heated slowly to 110–115°C under 4 m.m. Hg, pressure, it became colourless clear liquid, but even in this case, by a slightly sudden impulse, for instance if heat-supply was somewhat rapid, the yellow and yellowish-red metallic oxides appeared at once in the liquid and evolved gas containing carbondioxide. Keeping out of these changes and keeping this state of clear liquid, a small portion of liquid was distilled at near 115°C, which contained acetone, and the stage, where metallic oxide was born in proportion to temperature ascension, the distillates which had pungent odor and acetic acid, were obtained with CO_2 and other kinds of gas which had sweet smell and contained flammable one. Acetic acid anhydride, ketone and compounds of the type of mercury dialkyl were not detected or scarcely any in decomposition products. The formation of metallic mercury followed immediately after the appearance of oxides of mercury, and in this stage the decomposition were very rapid and violent, and as a consequence the products and decomposition processes were very complicate.[4]

Other experiments with $(C_2H_2O_2Hg)_5$,[5] which is proposed to be an intermediate compound in the thermal decomposition process of mercuric acetate, revealed lower decomposition temperature and an incomparably larger production of ketone than mercuric acetate.

Thermal decomposition of the compound, which is throught to posses the compo-

sition $C_6H_4CO_2Hg$  , began at 270-280°C, and, in proportion to temperature ascension, it deepened its yellowish colouring, and evolved gaseous products which contained a substance of sweet oder as well as a carbonyl compound, and then at near 320°C metallic mercury was born with violent reaction.[6]

It may be possible to presume that mercuric salts have a few analogous decarboxylation mechanism to that of other salts mentioned above, but, of course, there are many facts that can not be explained by this and older theories, and one which is to settle another mechanism is very desirable.

1) Ryonosuke Toyoda: Bull. Inst. Chem. Res., Kyoto Univ., **20**, 11 (1950); **21**, 32 (1950); **22**, 38 (1950).
2) Ryonosuke Toyoda: Unpublished.
3) Ryonosuke Toyoda: Unpublished.
4) Ryonosuke Toyoda: Unpublished.
5) Ryonosuke Toyoda: Unpublished.
6) Ryonosuke Toyoda: Unpublished.

4. The Action of Various Drugs in Case of Combined Use

a) The effects of antipyretics and hypnotics, especially in case of combined use with each other on the antipyretic action.[1]

Antipyretic action of pyramidon in the febered rabbit is slightly inhibited by small doses of veronal sodium or chloral hydrate, while the duration is much prolonged.

b) The effects of antipyretics and hypnotics, especially in case of combined use with each other on the blood sugar regulation.[2]

Pyramidon hyperglycaemia is inhibited by veronal sodium, luminal sodium, chloral hydrate or urethane in rabbits.

c) On the co-operative action of pyramidon and veronal.[3]

In mice the toxicity of pyramidon is greatly decreased by veronal, but synergism for analgetic action is not recognized. In rabbits, small doses of pyramidon prolong veronal narcosis, but large doses weaken.

d) Influence of pyramidon upon the veronal excretion in urine.[4]

Pyramidon inhibits veronal excretion in urine (in rabbits). Relations between this action and narcotic action as well as the mode of action were studied.

e) On the action of molecular compound and mixtures of pyramidon and veronal.[5]

Comparative studies of molecular compound and mixtures in various ratios of pyramidon and veronal regarding their toxicity, analgetic properties, influence of veronal excretion as well as their action on cultivated tissue (fibro blast) were examined. The mixtures are more powerful in inhibiting growth of cultivated tissue than molecular compound; as for other actions both drugs are almost the same.

f) The potentiating effects of various drugs upon the action of evipan and morphine.

It is pointed out that many local anesthetics, antihistaminics, antipyraetics, sedatives and some anticonvulsants potentiate the hypnotic action of evipan and analgesic action of morphine in mice however, with several exception. Drugs examined: Procaine, m-procain (p-amino dimethylaminoethylbenzoate hydrochloride), tutocain, stovain, alypyin, cocaine, nupercain, benadryl, 3015 RP, chlorobenadryl, antipyrin, aminopyrin, pyrabital (veramon), evipan, sodium bromide, tridion, myanesin and hustosil (guajacol glyceryl ether). Dimethylamino-radical on the side chain seems to develop a stronger analgesic action than diethyl-amino-radical.[6] Furthermore, some benzothiazole derivatives prolong the duration of evipan action, and potentiates morphine action in an additional combination of benadryl (by means of Haffner's method in mice). Drugs examined: 2-amino-benzothiazole, 2-amino-6-alkoxy-benzothiazoles, and 2-β-diethylaminoethylamino-6-alkoxy-benzothiazoles.[7]

g) On the action of antihistaminic drugs in case of combined use with barbital and comparative studies of the action of molecular compound and their mixture.[8]

The mode of action of molecular compound and mixtures concerning their toxicity, narcotic action as well as analgetic action in mice are almost similar. As to the antagonistic action of both drugs in toxicity, the mixture of chlorobenadryl and barbital is stronger than mixtures or molecular compounds of benadryl and barbital.

1) Tatsuo Minami: Bull. Inst. Chem. Res., Kyoto Univ., **12**, 173 (1941).
2) Tatsuo Minami: *Ibid.*, **12**, 183 (1941).
3) Tatsuo Minami: Folia Pharmacologica Japonica, **34**, 285 (1942).
4) Tatsuo Minami: *Ibid.*, **33**, 425, (1941).
5) Tatsuo Minami *et al*: *Ibid.*, **35**, 333, (1943).
6) Hajime Fujimura and Kenichi Nakajima: *Ibid.*, **45**, 57-95⅔ (1949); Bull. Inst. Chem. Res., Kyoto Univ., **25**, 36, 1951.
7) Hajime Fujimura: Folia Pharmacologica Japonica, **46**, 55 (1950); Bull. Inst. Chem. Res., Kyoto Univ., **20**, 69 (1950) (abstract of papers).
8) Hajime Fujimura and Kenichi Nakajima: Folia Pharmacologica Japonica, **46**, 127⅔, 1951.

5. Relations between Pharmacological Action and Chemical Structure

a) Pharmacological studies on various benzothiazol derivatives.[9]

Relations between chemical structure and action of various benzothiazole derivatives with respect to their toxicity in mice local anesthetic properties in rabbit's cornea and human skin as well as their action on smooth muscles in rabbit's intestines and frog's lungs were studied. Drugs examined: [6-amino-benzothiazolyl-(2)]-ethylether, [6-(β-diethylaminoethyl)-aminobenzo-thiazolyl-(2)]-butylether, 2-amino-benzothiazole-6-carboxylic acid-butylester, 2-amino-benzothiazole-6-carboxylic acid isoamylester, 2-amino-benzothiazole-6-carboxylic acid diethylaminoethylester, 2-amino-benzothiazole, 2-amino-6-methoxy-benzothiazole, 2-amino-6-ethoxy-benzothiazole, 2-amino-6-isopropoxy-benzothiazole, 2-[β-diethylamino-ethyl]-amino-6-methyl-benzothiazole, 2-[β-diethylamino-ethyl]-amino-6-methoxy-benzothiazole, and 2-[β-diethylamino-ethyl]-amino-6-ethoxy-

benzothiazole. Studies on these derivatives for practical uses are being made now.

b) On the action of various phenolalkylether derivatives.[10) 11)]

Regarding their toxicity in mice and oxytocic action in guinea pigs and rabbits, twenty seven derivatives were examined. It seems that [β-diethyl-amino-ethyl]-[2-formyl-6-allylphenyl] ether hydrochloride and 2-[β-diethylaminoethyoxy]-[3-allylbenzoic acid]-alkyl ester hydrochloride are most effective as an oxytocic.

c) On the action of some antihistaminic drugs.[12)]

The toxicity of chloro-benadryl (substitution of chlorine in 4-position of one phenyl radical of benadryl) is less, and the antihistaminic and the antianaphylactic action is stronger than benadryl (β-dimethylaminoethyl benzhydryl ether hydrochloride).

9) Hajime Fujimura: Folia Pharmacologica Japonica, **46**, 55 (1950).
10) Hajime Fujimura *et al*: *Ibid.*, **45**, 140, 158⅔, 1950.
11) Hajime Fujimura *et al*: *Ibid.*, **46**, 56⅔, (1950).
12) Hajime Fujimura *et al*: *Ibid.*, **46**, 60⅔, (1950).

6. The Action of Saccharina

From the pharmacological and toxicological standpoints the actions of various aromatic compounds, especially of saccharine series have been examined.

a) On the methemoglobin formation by various aromatic compounds, especially by their saccharine series.[13)]

b) Microscopic findings of blood and histological changes of internal organs after administration of phenetolcarbamide.[14)]

c) Pharmacological action of p-nitro-o-amino-phenyl-n-propylether.[15)]

13) Yoshinobu Tsujimura: Folia Pharmacologica Japonica, **45**, 49 (1950).
14) Yoshinobu Tsujimura: Unpublished.
15) Yoshinobu Tsujimura: Unpublished.

7. The Action of Papain Enzyme

It was found that the rongalite activates the enzymatic action of papain. And the mechanism of this reaction was studied.[16)]

16) Masashichi Yoshioka: Bull. Inst. Chem. Res., Kyoto Univ., **17**, 59 (1949); *Ibid.*, **20**, 68 (1950); *Ibid.*, **24**, 79 (1951).