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A Preliminary Investigation of Certain Purine Thiocyanate Complexes

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A PRELIMINARY INVESTIGATION

OF CERTAIN PURINE THIOCYANATE COMPLEXES

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INTRODUCTION

The purpose of the work has been to attempt to prepare some compounds of the purine group with regard to their possible use in cardiotherapy. Some of the purine derivatives such as caffeine, theobromine, and theophylline under certain conditions exert a stimulating effect on the heart, but under these conditions they usually also produce an undesirable rise in the blood pressure. It was desired to combine chemically the purine compounds with a structure or group which produced a dilating action on the blood vessels with the hope that such a combination might retain the desirable heart stimulating properties and decrease or completely nullify the undesirable effect on the blood pressure. For the first series of compounds it was decided to use the thiocyanates as the dilator group. If successful in preparing compounds, it was planned to have them tested on animals in order to determine their physiological effect.

HISTORICAL BACKGROUND

The first part of this section on the literature work will be devoted th the physiological effects of the thiocyanates and the purines. In this search only the abstract articles found in Chemical Abstracts and The Journal of the Chemical Society were studied since this part of the problem was only of general interest.

Thiocyanates:

In order to give a general idea of the work which has been done on the treatment of hypertension with thiocyanates and the results obtained, brief accounts of the articles follow in chronological order.

Takaoe (1) found that intravenous injections of sodium thiocyanate slowed the heart rate and lowered the blood pressure.

Behrens (2) learned from therapeutic experiments in man that sodium and potassium thiocyanate caused a decrease of blood pressure, but in the nine cases he worked with he thought the reduction was not sufficiently permanent to be of value as a medicinal.

Goldring and Chasis (3) studied the effects of thiocyanate therapy in hypertension on fifty patients. Their results showed that thiocyanate was 31% effective in lowering blood pressure and that toxic symptoms resulted in 17% of the group. In some of these cases there was no margin of safety between the toxic and the therapeutically active dose.

Healy (4) in his work on the therapeutics and toxicity of thiocyanates likewise found that doses of thiocyanate sufficiently great to reduce blood pressure may be toxic.

Maguire (5) treated four cases of high blood pressure with potassium thiocyanate. The blood pressure was reduced and there was a general feeling of well-being, except when the blood pressure was reduced too low. Discontinuation of the drug administration was followed by a return of hypertension to the original level.

Griffith and his colleagues (6) gave thiocyanate orally in sixteen cases of hypertension. Improvement resulted in ten of these cases.

Another group of doctors (7) administered doses of sodium thiocyanate to patients with uncomplicated vascular hypertension in sufficient quantities to maintain the blood thiocyanate concentration at 5-7mg.%. A lowering of the blood pressure was observed in every case and there were some transient toxic symptoms.

Wald, Lindberg, and Barker (8) studied the toxicity of thiocyanates. Their results showed that fatigue, nausea, dermatitis, disorientation, collapse, and death may result from thiocyanate therapy. In order to try to prevent these effects blood cyanate determinations should be used to control the administration

of thiocyanates.

A good summary of the use of potassium thiocyanate in the treatment of hypertension appeared in an abstract of an article in the Carolina Medical Journal (9). "Potassium thiocyanate is a safe vasodilator substance, if properly used, and lowers the systolic and diastolic pressures in the proper ratio. It can be made to lower the blood pressure gradually without the usual headaches associated with reduction by other means. In conservative doses potassium thiocyanate apparently does not produce anemia. It is present in the blood stream in a much larger percentage than any other known antipressor substance; it appears to be the natural agency with which to offset the effects of pressor substances. At times potassium thiocyanate exerts a cumulative action. There appears to be no tendency toward the development of a tolerance to the use of potassium thiocyanate."

From experiments on the intravenous injection of potassium thiocyanate into normal and hypertensive dogs Davis and Barker (10) believe that the depressor effect is produced as a result of a general vasodilation.

Herking (11) studied the effects of doses of alkaline thiocyanates which Biberfeld's text book had stated were non-toxic. Herking discovered however that these doses were toxic, and he suggested linking the thiocyanate to protein. He used a protein combination (rhodalside) in hundreds of cases and never observed toxic actions. He has taken out several patents (12) on his thiocyanate-protein preparations.

The results which Ellinger (13) found in studying the effects of anions on surviving arterial strips are shown below.

SCN^-, I^-, and NO_3^- — cause contraction

Br^- and Cl^- — no effect

NO_2^-, Ac.$^-$ salicylate,

citrate, tartrate, and benzoate — cause extension

The result shown for SCN^- ion is contrary to what has been said before, but of

course here the conditions are different than those in the living body and that may account for the disagreement.

All the work related so far has been in connection with inorganic thiocyanates. Taubmann (16) has carried out investigations on the effects of organic thiocyanates. His results follow. Aliphatic thiocyanates have a characteristic pharmacological effect consisting of convulsions, decrease in body temperature, and respiratory stimulation. Thiocyanates with double linkage are inactive, presumably because they are readily decomposed in the body; they still act as local irritants, however. Aniline thiocyanates are hemitoxic. In general, the organic thiocyanates are not pharmacologically analogous to the corresponding halogen derivatives.

Purines:

The purpose of this part of the report is to try to give information about the effect of purine derivatives, especially caffeine, theobromine, and theophylline, on the mammalian circulatory system. There are several factors which must be taken into consideration when studying these effects. Some of these considerations are enumerated below.

1. The compound that is being administered; whether or not it is given alone or in combination with one or more other compounds; and the dosage.

2. The method of administration, e.i. orally, intravenous or intra-arterial injections.

3. The part of the circulatory system being studied.

4. The condition of the animal or the isolated part of an animal before administration of the drug.

There may be other factors involved. Since all these conditions cannot be included in abstract articles, the report cannot be too detailed, but then it is not the purpose of the report to give a completely comprehensive account of

this work. It has been desired only to secure a general picture of the drug action of the purines on the circulatory system.

Heart Stimulation:

Yamamouti (18) in a paper on the pharmacological action of camphor and related drugs reports that caffeine stimulates the heart by both central and peripheral action (refering to the nerves), but large doses cause cardiac inhibition. The accompanying effect on the blood pressure is variable. If the central action predominates, the blood pressure rises; a dominant peripheral (refering to nerves surrounding the blood vessels) action causes a fall.

An abstract of a report by Charlier (18) tends to make the former paragraph a little clearer. He studied the variations in heart volume in the anaesthetised and vagotomised (surgical division of the vagus nerve which is either of the tenth pair of cranial nerves arising from the medulla and supplying branches to various organs) dog. Caffeine, theobromine and theophylline given by intravenous injection were found to increase the cardiac output, first by causing peripheral vasodilation and increasing the venous return and also by direct action on the myocardium (muscle wall of heart), causing a very energetic systole (contraction of the heart by which blood is forced onward). He stated that the three drugs are of about equal effect.

Flaum and Hessler (17) in their studies of caffeine, theobromine, and theophylline on experimentally damaged dog hearts state that the increased cardiac output per beat was not due to the increased coronary blood flow, but to a direct action on the heart muscle.

Vasodilation and Vasoconstriction:

1. Coronary vessels - All references of experimental work on the coronary vessels show that the three purine compounds under consideration all produce a vasodilating effect.

Heathcote (18) reports that all three drugs have an active vasodilator

effect on the coronary vessels, probably muscular in origin. Caffeine has the weakest action; theobromine, the strongest.

Several workers studied the effects of these purine derivatives on the coronary vessels of isolated hearts by the perfusion method. Savadskii (19a) found that caffeine solutions of concentrations 1:1000 to 1:10,000 [1:1000 to 1:3000 in one report (19b)] produced dilation. Iwai and Sassa (20) found the same effect for theophylline; and Trussevitsch (21), for theobromine (1:10,000).

2. Veins and arteries - Maloff (22) in his pharmacological investigations of isolated human veins found that various drugs affect the length and width of different veins differently. Caffeine, he found, causes constriction, and at times dilation. The reasons for this dual effect can be found in the factors mentioned at the beginning of this section of the report. Fujimori (23) shows how the effect of caffeine and theophylline on the blood vessels varys with concentration. Caffeine in 1% concentration produced slight contraction followed by dilation. In 0.01 to 0.1% concentration only dilation occurred. A 1% solution of theophylline contracted both veins and arteries. In 0.1% concentration, the arteries were slightly contracted and the veins were not altered. These experiments probably refer to perfusion experiments carried out on isolated blood vessels.

Beak (24), Ochiai (25), and Coppola (26) all report caffeine as a vasodilator, while Tremonti (27) reports it as a vasoconstrictor. If the conditions of their experiments were known, these seemingly contradictory statements would undoubtedly be found to be correct.

Effects on Blood Pressure:

The dosage given seems to be of great importance in regard to the effect of caffeine on the blood pressure. Pilcher has done quite a large amount of work on the action of caffeine on the mammalian circulation. He reports (28) that in small doses caffeine increases the cardiac tone and produces a vascular relaxation or dilation which is peripheral in origin since the vasomotor center

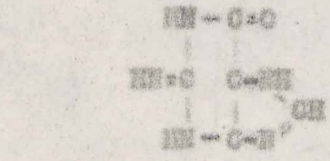
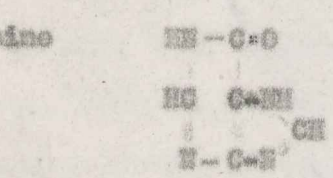
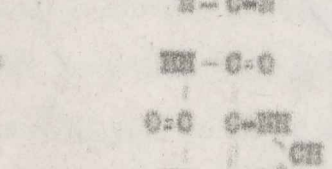
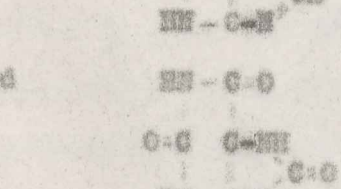
is itself stimulated. A rise in blood pressure is also usually found. Larger doses decreased the cardiac tone, produced further vascular dilation, and lowered the blood pressure. In other reports (29) and (30) he gives the dosages and their effects. For doses up to 20 mg./kg. of body weight the heart rate increases and there is a moderate rise in blood pressure. With doses from 20 to 150 mg./kg. the blood pressure falls to a constant level of 60 to 70 mm. depending upon the original pressure. The heart rate is increased and the central vasomotor stimulation persists. Additional doses produced no further effects until death occurred by cardiac paralysis. The fatal doses ranged from 57 to 800 mg./kg.; the average being 175 mg. Certain physiological effects observed indicated that caffeine depressed the peripheral vasomotor system. Experiments on excised organs showed that caffeine produced this effect by preventing constriction. These doses just spoken of must have been given orally (although the abstract did not definitely say that) since in one of them Pilcher states that intravenous injections of caffeine cause a slow and slight rise in blood pressure followed by a sharp fall. Something which Pilcher observed shows how the method of administration may affect the effect of caffeine. A fall in blood pressure followed rapid intravenous injection of the drug. This did not occur with other methods of administration.

Horst and his colleagues (21) and (32) in their studies on caffeine and coffee confirm the results of Pilcher in that a rise in blood pressure was caused by small doses. Martinetti and Perconi (33) state from their study of the action of caffeine on healthy and hypertensive subjects that an immediate, moderate rise of pressure occurs for intevenous injections of 0.2 to 0.5 g. They were probably referring to the immediate effect that Pilcher mentioned. Later Martinetti reported (34) that approximately these same dosages of caffeine had a variable effect but that the general tendency was to cause a transient fall in blood pressure. Taylor (35) mentions a drop in both systolic and diastolic blood pressures for average doses of 0.7 g. of caffeine, which is

below Filcher's 20 mg./kg., but here we do not know his method of administration.

Influence of Constitution of Purine Compounds on Their Physiological Effect:

Dengres and Berleans (36) studied the influence of chemical constitution on arterial pressure. Using doses of 20 to 30 mg./kg. body weight injected intravenously, their results are shown in the following table.

Compound	Structure	Degree of Oxidation	Effect on Blood Pressure
Guanine		1 oxygen atom	Lowered pressure 30 to 60mm. Hg (dogs and rabbits)
Hypoxanthine		1 oxygen atom	Raised pressure 7 mm. (rabbits)
Xanthine		2 oxygen atoms	Raised pressure 16 mm. (rabbits)
Uric acid		3 oxygen atoms	Raised pressure 26 mm. (rabbits)

It should be noted how, from guanine to uric acid, the chemical molecule is transformed, first by the removal of the amino group, then by progressive oxidation with increase of acid quality to which, they claim, the hypertensive action of the oxypurines is due.

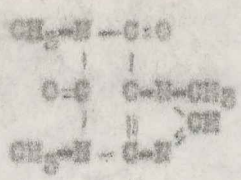
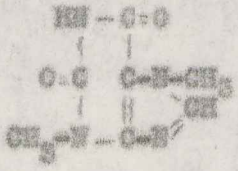
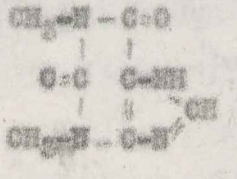
These same workers (37) studied the influence of molecular weight and constitution on the toxicity of some organic nitrogenous compounds included in which were caffeine and theobromine. They came to the conclusion that the toxicity of organic nitrogenous compounds of analogous constitution decreases as the molecular weight decreases.

It appears that a chemical stimulant or depressant may exert its action due to one or more molecular groups in its structure; and that one atom or group of atoms may modify the physiological action of other atoms or groups of atoms in the same molecule.

Pickering (38) has studied the effect that the introduction of a chlorine atom and the cyano group separately into the caffeine molecule has on the physiological action of caffeine. Studying the effects of chlorocaffeine on hearts of embryo chicks, frogs, and human beings, he found that the chlorine atom considerably modifies the physiological action of caffeine. Chlorocaffeine produces far less tonic contraction than caffeine itself. The three methyl groups in caffeine tend to induce tonic contraction of the heart muscle and the chlorine atom tends to produce an atonic condition. The cyano group in cyanocaffeine overpowers the physiological action of the three methyl groups, and the compound acts more like a cyanogen derivative than a xanthine derivative. There is no evidence of tonic contraction at all. The compound is almost immediately fatal to chick and frog hearts, the heart dying in extreme diastole. In these experiments the possibility of living tissue decomposing chlorocaffeine and cyanocaffeine into free chlorine, cyanogen, and caffeine respectively was not overlooked, but tests failed to give evidence of these substances.

The following table (39) gives a little information about compounds referred to in this literature work and used in the experimental work. It will be useful as a reference.

Compound	Structural Formula	Empirical Formula	M.W.	M.P.
Thiocyanic acid	H-S-C≡N	HSCN	59.00	5° ± 0
Potassium thiocyanate	K-S-C≡N	KSCN	97.17	172.8°
Urea	$H_2N-\overset{O}{\overset{\|}{C}}-NH_2$	CH_4N_2O	60.06	132.7°

Compound	Structural Formula	Empirical Formula	M.W.	M.P.
Caffeine		$C_8H_{10}O_2N_4$	194	235° dry subl. 180°
Theobromine		$C_7H_8O_2N_4$	180	330° (sealed tube) subl. 290°
Theophylline		$C_7H_8O_2N_4$	180	269-72°

Complexes of Urea and Related Substances:

Since the purine derivatives interested in contain two urea residues, it was planned to do some preliminary work with urea. For this reason a search of the literature was made of urea and its related substances, thiourea and guanidine.

Acid Salts of Urea:

Urea is a weak base, having an ionization constant of $1.5 \cdot 10^{-14}$ at 25°C (39). DuFoit's work (40) on acid salts of urea showed that the following salts are stable in contact with their solutions at temperatures of about 20°.

Acid	Salt
Oxalic	$(NH_2 \cdot CO \cdot NH_2)_2 \cdot (CO_2H)_2$
Acetic	$(NH_2 \cdot CO \cdot NH_2) \cdot (HOAc)_2$
Hydrochloric	$(NH_2 \cdot CO \cdot NH_2)_2 \cdot HCl$ and $(NH_2 \cdot CO \cdot NH_2) \cdot HCl$
Nitric	$(NH_2 \cdot CO \cdot NH_2) \cdot HNO_3$
Sulfuric	$(NH_2 \cdot CO \cdot NH_2)_2 \cdot H_2SO_4$ and $(NH_2 \cdot CO \cdot NH_2) \cdot H_2SO_4$

Thiourea Complexes:

Natiko (41) has investigated some of the compounds which thiourea forms with metallic salts. Compounds such as the following were obtained; $(NH_2 \cdot CS \cdot NH_2) \cdot CuCl$, $(NH_2 \cdot CS \cdot NH_2)_2 \cdot CuCl$, and $(NH_2 \cdot CS \cdot NH_2)_3 \cdot CuCl$. From the chemical reactions of these compounds the author believes them to be true chemical compounds and not merely molecular compounds. Salts of Ba, Mg, Co, Ni, Mn, Fe^{++}, and Cr do not show any change in reactions of their neutral or acid solutions when mixed with thiourea, but solutions of $HgCl_2$, $Hg(CN)_2$, AgCl, $AgNO_3$, $CoSO_4$, $ThSO_4$, and lead chlorides did show a change and therefore indicated the formation of double compounds.

Reynolds (42) has prepared ammonium halide salts of the type tetrathiourea ammonium halide, $(NH_2 \cdot CS \cdot NH_2)_4 \cdot NH_4X$. An example of the preparation of such a compound follows.

Tetrathiourea ammonium bromide is prepared by the direct action of ammonium bromide on thiourea. The best results were obtained with the following procedure. 10 g.(1mole) ammonium bromide were dissolved in the smallest quantity of hot alcohol necessary for its solution. This solution was then added to a boiling and nearly saturated alcoholic solution containing 30.4 g.(4moles) of thiourea. The mixture was allowed to boil for five minutes and then cooled. Aggregations of radiating crystals soon began to form, and when cold the whole formed a nearly solid, white, crystalline mass. The reaction product was obtained in a pure state after being drained, pressed and then recrystallized from alcohol. Some of the properties of this substance follow:

m.p. 178-4°, decomp. 178-80°

Easily soluble in boiling absolute alcohol. Much less soluble in cold alcohol.

Almost insoluble in ether, chloroform, and benzene.

Easily soluble in water (when pure, its aqueous solution can be boiled violently for half an hour without undergoing decomposition;

but if impure, partial decomposition occurs.)

Is easily decomposed by boiling acids and alkalies and is readily desulphurized by alkaline lead tartrate.

The corresponding iodide (m.p. 186°, decomp. 190°) and the chloride (m.p. 184°) were also prepared. In all three cases experiments were conducted using one, two, and three moles of thiourea respectively in order to see if other compounds were formed. In all cases, the amount of compound obtained indicated that only the tetrathiourea compound was formed under the experimental conditions.

C. Carrara (43) claims to have prepared the compound thiourea ammonium thiocyanate, $(NH_2\ CS\ NH_2)\ NH_4SCN$, by dissolving equal weights of thiourea and ammonium thiocyanate in water and allowing the solution to evaporate. He gives as the properties: m.p. 144°; 25 parts dissolve in 100 parts of water at 25°; and the compound dissociates in aqueous solution.

In connection with the work mentioned previously Reynolds conducted experiments using substituted thioureas and ammonium halide salts and thiourea and substituted alkyl ammonium halide salts. This work was done in order to try to determine which of the following structural formulas was the correct molecular constitution of thiourea.

$$H_2N\text{-}\overset{S}{\overset{\|}{C}}\text{-}NH_2 \qquad\qquad NH\text{=}\overset{SH}{\overset{|}{C}}\text{-}NH_2$$

The experimental results seem to favor the unsymmetrical form. The tetrathiourea ammonium bromide compound would therefore become $[NH_2(SH)CNH_2]_4NBr$, but whether the union is affected by breaking the double bond between the carbon atom and the imino group or by the change of valency of the latter remains undetermined. Reynolds seems to favor the latter mechanism of union.

Guanidine Thiocyanate:

Krall (44) claims to have prepared guanidine thiocyanate by the fusion of a mixture of thiourea and ammonium thiocyanate. Fusions were started with an equilibrium mixture of 28% thiourea and 72% ammonium thiocyanate and were

carried out between temperatures of 170° and 255°. The essential changes were believed to be those shown in the equations below:

$$NH_2\text{-}\overset{S}{C}\text{-}NH_2 \longrightarrow H_2S + NH_2\text{-}C{\equiv}N$$
cyanamide

$$NH_2\text{-}\overset{N}{C} + NH_4SCN \longrightarrow NH_2\text{-}\overset{NH}{C}\text{-}NH_2 \cdot HSCN$$
guanidine thiocyanate

Crude guanidine thiocyanate was extracted from the fusion mixture by treatment with water and then treatment of the water residue with absolute alcohol. Pure guanidine thiocyanate was obtained by recrystallization from ethyl acetate. The product is colorless and gives off ammonia when heated.

An experiment was performed using potassium thiocyanate in place of the ammonium salt. A mixture of thiourea hydrochloride and potassium thiocyanate were fused. Hydrogen sulfide and carbon disulfide were evolved, but no guanidine was found in the fusion mixture. Much of the thiocyanic acid formed had polymerized.

Caffeine and Theobromine Complexes:

Caffeine is weakly basic. Its ionization constant as an acid is less than $1\cdot10^{-14}$ at 25°C and as a base is $4.1\cdot10^{-14}$ (39). There seems to be some disagreement in the literature about the existence of some of the acid salts of caffeine. Schmidt (45) states that salts of caffeine are formed only when concentrated acids react with the base. He claims to have obtained the following salts.

Salt	Crystal Shape	Color	Remarks
Caffeine hydrochlorides			
$C_8H_{10}N_4O_2 \cdot HCl \cdot 2H_2O$	prismatic	colorless	-----
$C_8H_{10}N_4O_2 \cdot HCl$	-----	-----	dry HCl gas + caffeine
Caffeine hydrobromide			
$C_8H_{10}N_4O_2 \cdot HBr \cdot H_2O$	-----	colorless	-----

Salt	Crystal Shape	Color	Remarks
Caffeine hydroiodides			
$C_8H_{10}N_4O_2 \cdot HI$	-----	-----	-----
$C_8H_{10}N_4O_2 \cdot 2HI$	-----	-----	-----
Caffeine nitrate			
$C_8H_{10}N_4O_2 \cdot HNO_3 + H_2O$	thick needles	yellow	-----
Caffeine sulfate			
$C_8H_{10}N_4O_2 \cdot H_2SO_4$	needles	colorless	crystallized from hot alcoholic soln.
Caffeine acetate			
$C_8H_{10}N_4O_2 \cdot (C_2H_4O_2)_2$	needles	colorless	decomposed by exposure to air
Caffeine normal butyrate			
$C_8H_{10}N_4O_2 \cdot C_4H_8O_2$	needles	white	-----
Caffeine isovalerate			
$C_8H_{10}N_4O_2 \cdot C_5H_{10}O_2$	needles	colorless	-----

All these salts are decomposed by water, ethyl alcohol, and ether and wholly or partially decomposed by heating to 100° C to give caffeine and the acid.

Tarret (46) disagreed with most of this previous work. He states that caffeine, owing to its weak basic properties and neutral reaction, does not neutralize even the smallest trace of acid; and that it does not form salts with organic acids. Acetic, valeric, lactic, and citric acids merely dissolve the caffeine, and upon cooling the solutions the caffeine separates out. With mineral acids, he agrees that caffeine does form salts. Because of the unstable character of these salts, however, they are useless for hypodermic injections. He found that caffeine formed complexes with the sodium salts of benzoic, cinnamic, salicylic, acetic, lactic, citric, sulfuric, and hydrochloric acids which were similar to caffeine and were very soluble in water. These salts were prepared by treating caffeine with its equivalent of the sodium salt dissolved in a small quantity of water. By means of these compounds caffeine could by used for

hypodermic injections.

Since there had been this disagreement Biedermann (47) examined caffeine and its principal salts. By dissolving caffeine in the appropriate acid and evaporating the solution over potassium hydroxide he reported obtaining all the salts that Schmidt had obtained, with the exception of the anhydrous hydrochloride and the normal butyrate. In addition he obtained the following salts.

Aurochloride	$C_8H_{10}N_4O_2 \cdot HCl \cdot AuCl_3 + 2H_2O$
Platinochloride	$(C_8H_{10}N_4O_2)_2 \cdot H_2PtCl_6$
Formate	$C_8H_{10}N_4O_2 \cdot CH_2O_2$
Benzoate	$C_8H_{10}N_4O_2 \cdot C_6H_6O_2$
Chlorides	$C_8H_{10}N_4O_2 \cdot 4HCl$
	$C_8H_{10}N_4O_2 \cdot HCl$
Periodide	$(C_8H_{10}N_4O_2 \cdot HI \cdot I_2)_2 \cdot 3H_2O$

Heilbron (48) states that theobromine is faintly basic, having an ionization constant as a base of 11.1×10^{-11} at 25° and 4.6×10^{-14} at 40° but that it forms more stable salts with bases than with acids. Paul (49) in his work on caffeine and theobromine and their salts gave for ionization constants of theobromine the following:

For dissociation into $C_7H_6O_2N_4 \cdot OH^-$ and H^+ K 1.38×10^{-9} at 18°

For dissociation into $C_7H_6O_2N_4 \cdot H^-$ and OH^- K 1.8×10^{-14}

Schmidt and Pressler (50) reported having prepared the following acid salts of theobromine by dissolving theobromine in concentrated acids. These salts resemble those of caffeine in their instability.

Salt	Crystal Shape	Color
Hydrochloride		
$C_7H_6O_2N_4 \cdot HCl + H_2O$	plates	colorless

Salt	Crystal Shape	Color
Hydrobromide		
$C_7H_8O_2N_4 \cdot HBr + H_2O$	needles	colorless
Platinochloride		
$(C_7H_8O_2N_4)_2 \cdot H_2PtCl_6 + 4H_2O$	----	----
Aurochloride		
$C_7H_8O_2N_4 \cdot HAuCl_4$	needles	yellow
Sulfate		
(varying composition)	----	colorless
Nitrate		
$C_7H_8O_2N_4 \cdot HNO_3$	----	----
Acetate		
$C_7H_8O_2N_4 \cdot C_2H_4O_2$	voluminous precipitate	white

Caffeine upon ordinary treatment with dilute alkalies is decomposed (51). With very gentle action the caffeine merely takes on one mole of water and is converted to an acid called caffeidine-carboxylic acid, $C_8H_{12}O_3N_4$. Theobromine acts quite differently. It reacts with both alkalies and alkaline earths like an acid, forming definite salts.

The sodium salt of theobromine, $C_7H_7O_2N_4Na$, is obtained by adding theobromine to a sodium hydroxide solution in such a quantity that a portion remains undissolved after long standing, and evaporating the filtrate under an air pump. Milk-white crusts and rings form; these have no crystalline structure. The salt is extremely soluble in water and has a strong alkaline reaction. It absorbs carbon dioxide from the air and is thereby decomposed. In this work Haly and Andreasch also report the preparation and properties of the barium salt, $(C_7H_7N_2O_4)_2Ba$.

Mengor and Weller (52) have prepared double salts of theobromine.

In their patent they state that double salts of theobromine, which are more soluble in water than free theobromine, have previously been manufactured by combining theobromino-sodium with molecular proportions of alkali salts of organic acids. Double salts with sodium and lithium formate, acetate, lactate, benzoate, and salicylate have thus been obtained. They discovered double salts which are formed by combining in solution molecular proportions of theobromino-sodium and a halogen salt such as the sodium or potassium chlorides, bromides, or iodides. In giving the advantages of these new double compounds they state: "all these compounds have the advantage of being readily soluble in water and the sodium chloride double salt is distinguished by its high contents in theobromine, which is higher than in any other similar preparation, and it has, in therapeutics, the further advantage that the theobromine can act without the addition of an acid foreign to the human body. Other salts, for instance the sodium bromide and sodium iodide double salts, contain, in addition to theobromine, other substances which in this combination have a special beneficial therapeutical action. Examples are given for preparation of two of the salts. The salts are white powders which have an alkaline reaction and a bitter taste. They are readily soluble in water, dilute alkalies and dilute alcohol, insoluble in ether, absolute alcohol and benzene, and are decomposed by acids.

Kaufmann (53) has prepared complex salts from alkaline earth halides and thiocyanates and methylxanthines. The following is a list of compounds he claims to have prepared.

Theobromino-calcium·$CaBr_2$·6 (or 9) H_2O

Theobromino-calcium·CaI_2·10 H_2O

Theobromino-potassium·$Ca(SCN)_2$·5 H_2O

Theobromino-strontium·$SrBr_2$·5 H_2O

Theobromino-strontium·SrI_2·9 H_2O

Theobromino-strontium·$Sr(SCN)_2$·6 H_2O Hygroscopic

$(Caffeine)_2 \cdot Ca(SCN)_2 \cdot 2\ H_2O$

$(Caffeine)_2 \cdot Sr(SCN)_2 \cdot H_2O$

Gruter (56) has prepared a readily soluble, neutral, nondeliquescent product by treating caffeine with more than the equivalent amount of thiocyanic acid and ethylenediamine or of the ethylenediamine salt of thiocyanic acid. The product was used for aural, rectal, intramuscular and intravenous injections.

Paul and Ruhl (55) studied the constitution of medicinally applied purine derivatives in solution. They found that combination type complexes occur whose components behave in aqueous solution as though they were free and uncombined. Existing bonds in the sense of the field-valence theory are manifestly of so little force that they no longer appear on introduction of the substance into water.

APPROACH TO EXPERIMENTAL WORK

Since caffeine, theobromine and theophylline contain two urea residues, it was decided to do some preliminary work with urea in order to see if and how a double salt between urea and potassium thiocyanate might be prepared. If successful in this work, some knowledge which might be of value in the future work on the purine derivatives might be obtained. It was also wished to learn what effect the urea in such a combination would have on the toxicity and the dilating action of the potassium thiocyanate.

The first method of approach, suggested by Krall's work on guanidine thiocyanate, was to be a fusion of equimolecular proportions of urea and potassium thiocyanate. If this proved unsuccessful, or if successful and time permitted, it was planned to try the method of preparation used by Reynolds in his preparation of thiourea complexes with the ammonium halide salts.

Based on the results of this work, an attempt to prepare a complex between caffeine and potassium thiocyanate was to be made. Since, as will be seen later, this attempt proved unsuccessful, it was decided to try to prepare a thiocyanate salt of caffeine.

The third part of the work was to attempt to prepare the double salt theobromine-sodium potassium thiocyanate by the method used by Mosgor and Weller in their preparation of the alkali halide double salts.

RESULTS

The double salt urea potassium thiocyanate was prepared. Its properties are given in the experimental section.

The experimental work of trying to prepare a double salt between caffeine and potassium thiocyanate or thiocyanic acid proved to be unsuccessful. Since Kaufmann (33) was able to prepare double salts of caffeine with calcium and strontium thiocyanates, it is believed that a double salt with the potassium salt can be prepared by use of a method different from that tried. The acid salt can also probably exist since thiocyanic acid is a fairly strong acid, having an ionization constant of 10^{-1} (36). For comparison, the ionization constants of several organic acids with which caffeine forms salts are given below. (39)

Acid	K
Acetic	1.86×10^{-5}
Benzoic	6.6×10^{-5}
Formic	2.14×10^{-4}
Butyric	1.48×10^{-5}

Since thiocyanic acid polymerizes very rapidly above 0° to a solid yellow

substance, the acid salt with caffeine would probably be stable only at low temperatures and would probably decompose at room temperatures.

Due to lack of time and information, the work done in trying to prepare theobromine-sodium potassium thiocyanate was not carried far enough to give either a negative or a positive result as to whether or not a compound was formed.

SUGGESTIONS FOR FURTHER INVESTIGATION

The work with the theobromine-sodium double salt and the caffeine double salt should be continued. Before doing any more experimental work it would be wise to look up Kaufmann's (33) original paper to learn how he prepared and characterized his purine double salts. His paper should give some valuable information. If future results are successful, a double salt of theophylline should also be prepared.

After this work has been completed it would be interesting to prepare thiocyano compounds of urea, the pyrimidines and the purines, and have them tested for their physiological effect. This might give some valuable results. As mentioned in the historical background Deegres and Dorleans (see page 6.) stated that the hypertensive effect of the purine derivatives was due to the acid quality of the molecule which depended upon its degree of oxidation. If an oxygen atom were replaced with a thiocyano group, this might decrease the hypertensive effect by decreasing the degree of oxidation and the thiocyano group might also act as a dilator. These thiocyano compounds should not be very difficult to prepare.

If all this work should prove unsuccessful or even if successful, it might be interesting to work with some other dilating compound or group. Suggestions for such work can be found in Goldblatt, Kahn, and Lewis's article on all the methods used at the present time in the treatment of hypertension.

EXPERIMENTAL

Urea Potassium Thiocyanate, $(NH_2 \cdot CO \cdot NH_2) \cdot KSCN$:

Preparation and Purification:

A typical example of the method used in preparing this double salt follows. 60.1 g. (1 mole) of urea and 97.2 g. (1 mole) of potassium thiocyanate were mixed and the mixture was slowly heated. At approximately 136° most of the crystals had fused. The temperature was further raised to 150°. A small amount of fumes were given off. Their odor contained some ammonia but was not very strong. The molten mixture had a light dull yellow color. In the fusion no sudden rise of temperature was observed. The liquid was allowed to solidify and then broken up.

The following table contains approximate relative solubilities of potassium thiocyanate, urea, and the crude fusion product. In the determination of all solubilities in this experimental work a small amount of solid, approximately 0.1 g., was added to 2 or3 cc. of solvent. Solubility is indicated by a plus sign and insolubility by a negative sign.

Solvent	KSCN	Urea	Fusion Mixture
H_2O (cold)	+	+	+
EtOH (cold)	-	+	+ partially
(hot)	+	+	+ much more than in cold
Acetone (cold)	+	-	+ partially
(hot)	+	+ partially	+ much more than in cold
Benzene (cold)	-	-	-
(hot)	-	-	- mixture melts forming 2nd heavier layer
Petroleum Ether (cold)	-	-	-
(hot)	-	-	-
$CHCl_3$ (Cold)	-	-	-
(hot)	-	-	-

Solvent	KOH	Urea	Fusion Mixture
EtAc (cold)	- EtAc turns cloudy white & then pink	- EtAc turns cloudy white	- EtAc turns cloudy pink
(hot)	-	-	-
5% HCl (cold)	+	+	+
5% NaOH (cold)	+	+	+

From this solubility data it was decided to try to recrystallize any product that might have been formed from alcohol and from acetone. Needle shaped, white crystals having melting points of 99° - 101.5° (corrected) as determined by means of the Thiele tube were obtained from both of these solvents. The melting point of the fusion mixture was 96.5° - 100.5°. At the lower temperature the column of crystals sinter to form a cloudy white mass which is melted to a clear liquid at the upper temperature. With some of the recrystallisation products the upper melting point temperature was 102°. This 2.5 to 3 degree melting point range was not decreased by second and third recrystallisations from the solvents.

In three out of eleven recrystallisations from alcohol trouble was experienced. In two of these the product started melting at 99° but was not completely melted even at 110°. The third product melted in the range between 165° to 175°. Evidently a splitting of the double salt occurred giving in the first two cases a mixture of the double salt, urea , and potassium thiocyanate. The third product gave off no ammonia when heated with solid sodium hydroxide; and the melting point of potassium thiocyanate is 172.5°. These facts indicate that in the third case the decomposition progressed further and a mixture composed mainly of potassium thiocyanate crystallised from the alcohol. This decomposition may have been due to the water present in the alcohol. Some of the product with a 2.5° melting point range was refluxed with distilled water for about one half hour. The solution was then evaporated to dryness in the drying hood and the solid was recrystallized from alcohol. The melting point

range of the product was 166° to 175°.

An attempt was made to prepare the double salt according to the method used by Reynolds (see page 11.). Equimolecular amounts of urea and potassium thiocyanate were dissolved in hot alcohol. The two solutions were then mixed and heated for approximately five minutes. Since no crystals deposited upon cooling the solution, it was partially evaporated on a water bath. The crystals obtained from the solution melted in the range 168° to 172° showing that they consisted of a mixture containing predominantly potassium thiocyanate.

Analytical:

The solubilities have been recorded in the previous section. An aqueous solution of the product is neutral to litmus.

The melting point of the urea potassium thiocyanate has also been given in the previous section. Its boiling point is around 180°. At this temperature the liquid has a light green color and gives off ammonia.

Upon heating the product with solid sodium hydroxide, ammonia is evolved. This indicates the presence of urea. An aqueous solution of the double salt gives the characteristic red color of ferric thiocyanate when treated with ferric chloride. The residue remaining upon ignition of the product gave an alkaline test. This gave proof of the presence of potassium.

The Kjeldahl method was used in determining the nitrogen content of the product. No difficulties were experienced in the determinations, the results of which are given in the following table.

Sample	Weight of Sample	Weight of N	% N
1	0.0615 g.	0.0154 g.	25.2 %
2	0.0669	0.0173	25.8
3	0.0686	0.0166	24.1

Theoretical % N 25.7 %

Average experimental % N 25.7 %

The difference of 1.0 % in these percentages is within the accuracy of the Kjeldahl apparatus used.

The crystals of urea potassium thiocyanate, urea, and potassium thiocyanate as formed by evaporation of a drop of an alcohol solution of these substances on a glass plate were studied briefly under the microscope. Not a great deal could be determined, but it seemed that the crystals of the double salt were different from those of urea and potassium thiocyanate. This work also showed that the double salt product was more hygroscopic than potassium thiocyanate.

Work with Caffeine:

Attempt to Prepare Caffeine Potassium Thiocyanate:

Equimolecular amounts of caffeine and potassium thiocyanate, 2 g. and 1 g. respectively, were fused. At 100° small spots of purple color had appeared throughout the mixture. At 180° the mixture had partially melted into a thick pasty mass. The temperature was raised to 200° at which all the crystals had melted. Upon allowing the solution to cool with stirring, crystals began to appear at 195° . The solid had a slight pinkish tinge and upon grinding gave a white powder. It had an odor similar to cabbage.

Solubilities of caffeine and the fusion product are given in the following table.

Solvent	Caffeine	Fusion Mixture
H_2O (cold)	-	- slightly sol.
(hot)	+	+
EtOH (cold)	-	-
(hot)	+	+
Acetone (cold)	-	-
(hot)	- More than in cold	+
Benzene (cold)	-	-
(hot)	- more than in cold	- more than in cold

Solvent	Caffeine	Fusion Mixture
Petroleum Ether (cold)	-	-
(hot)	-	-
Et_2O (cold)	-	-
(hot)	-	-
$CHCl_3$ (cold)	+	-
(hot)	+	-
CCl_4 (cold)	-	-
(hot)	-	-
Glacial acetic acid (cold)	-	- pink soln.
(hot)	+	+
EtAc (cold)	- turns cloudy	- turns cloudy
(hot)	-	-
5 % NaOH (cold)	-	-
(hot)	+	+
5 % HCl (cold)	+	+
(hot)	+	+

The crude fusion product gave a thiocyanate test with ferric ion, but products obtained from benzene and acetone recrystallizations contained no thiocyanate.

Attempt to Prepare Caffeine Thiocyanate, $C_8H_{10}O_2N_4 \cdot HSCN$:

Two methods were tried namely 1 the mixing of acetone solutions of thiocyanic acid and of caffeine, and 2 the fusion of caffeine hydrochloride and potassium thiocyanate.

Method 1, Using Acetone Solutions of Caffeine and Thiocyanic Acid:

Potassium chloride is insoluble in acetone. An acetone solution of thiocyanic acid was prepared by dissolving potassium thiocyanate in acetone and then passing dry hydrogen chloride through this solution. The potassium

chloride which precipitates is then filtered off leaving a slightly pinkish solution. The color is probably due to a trace of ferric salt in the filter paper.

A solution of thiocyanic acid was prepared in this way by using about 2 g. of potassium thiocyanate. 2 g. of caffeine were dissolved in hot acetone and the two solutions were mixed and set aside to cool. Only 1 g. of potassium thiocyanate is required to give an amount of thiocyanic acid equivalent to 2 g. of caffeine, but an excess was used. White, needle-like, spherulite crystals were formed. These were filtered off and washed with cold acetone. They gave no test for SCN^- ion. The acetonefiltrate was evaporated. A mixture of yellowish crystals and whitish coal was left which gave a positive test for SCN^- ion and had an onion or garlic odor. The yellow color and the odor were probably due to polymerized thiocyanic acid. Solubilities of this residue were determined and some of it was recrystallized from alcohol. The crystals obtained, however, contained no thiocyanate.

Method 2. Fusion of Caffeine Hydrochloride and Potassium Thiocyanate:

Equimolecular portions of caffeine hydrochloride (1 g.) and potassium thiocyanate (0.4 g.) were mixed and slowly heated. Between 80° and 90° with stirring the mixture began to turn a light yellow. This color also seemed to be concentrated at the surface of small crystals of potassium thiocyanate. The temperature was carried to 230°. As the temperature rose the mixture turned to a darker yellow and finally to a brownish yellow mass. At 210° the mixture was a runny mass, but it never became a liquid. Fumes that were given off had a weak odor and gave a test for hydrogen sulfide. Yellow droplets also condensed on the walls of the test tube. The mixture was cooled and pulverized.

This fusion product was insoluble in all the solvents worked with. It was partially soluble in hot alcohol, acetone, benzene, chloroform, carbon tetrachloride and glacial acetic acid since upon standing for a few days clear needle crystals were formed.

A qualitative analysis of the fusion product was performed. A positive test was obtained for nitrogen, but negative tests were obtained for sulphur and chlorine. Further work on the mixture was therefore not undertaken.

Work on Theobromine:

Attempt to Prepare Theobromine-Sodium Potassium Thiocyanate, $C_7H_7O_2N_4Na \cdot KSCN$:

Following the method used by Mauger and Weller 1.8 g. (0.01 mole) of theobromine was dissolved in a solution of 0.4 g. (0.01 mole) of sodium hydroxide in about 3 or 4 cc. distilled water. This solution was heated in order to dissolve most of the theobromine. To this was added 1.0 g. (approximately 0.01 mole) of potassium thiocyanate. Upon solution of this, the solution was filtered and evaporated. The residue was ground.

The substance was soluble in water and hot acetic acid, probably being decomposed by the latter, and insoluble in all the other ordinary solvents. It was very slightly soluble in hot acetone and hot dry alcohol. Upon cooling these solutions a voluminous white precipitate was formed. The water solution was basic to litmus.

Time did not permit the work to be carried further.

SUMMARY

The double salt uron potassium thiocyanate was prepared and was sent away to be tested for its physiological action. Preliminary tests indicate that the compound is much less toxic than potassium thiocyanate itself, but the hypertension effects have not yet been determined.

The work on the preparation of caffeine potassium thiocyanate, caffeine thiocyanate, and theobromine-sodium potassium thiocyanate, as far as carried, proved to be unsuccessful.

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