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Summary

Acetolysis of 2′,3′,5′-tri-O-benzoylguanosine with glacial acetic acid, acetic anhydride, and sulfuric acid gave rise to 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in a fairly good yield (overall yield from guanosine was 50%). This method will afford a most convenient procedure for preparing 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose which is a key intermediate in the synthesis of ribonucleosides and ribonucleotides.

The direct acetolysis is one of the rare cases in which N-glycosides are easily acetolyzed without cleavage of the furanoside ring.

Some observations on hydrolysis and acetolysis of 2′,3′,5′-tri-O-benzoylguanosine were also described.

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67. Mitsuru Uchiyama*1 and Tyunosin Ukita*2 : Protection of Mammalia from the Poisoning of Radioactive Strontium. IV.*3 Aluminum Citrate.

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It was pointed out by Copp, *et al.*[1] that feeding of mice with low-phosphorus diet results in increasing excretion of administered strontium. In the previous paper of this series,[2] the effect of dihydroxyaluminum aminoacetate (DAA) on elimination of radioactive strontium was reported. In the case of DAA-treatment, the aluminum contained in DAA fed to mice with normal food was liberated in gastrointestinal canal and made the phosphates in the food insoluble to inhibit its gastrointestinal absorption. By this process the food containing DAA caused the same result as a low-phosphorus diet.

On the other hand, it has been indicated[3] that some kind of organic acids, i.e. citric and acetic acids, stimulated the excretion of strontium even when they were administered orally. Further, several organic acids, i.e. citric, tricarballylic, and lactic acids, are known to have the ability of forming chelate compounds with strontium and of stimulating excretion of the metal.[4] It may be possible to expect an additional effect in this line for aluminum salts of these acids. This paper deals with the result of the research on above-described effect of these salts.

Aluminum citrate, aluminum tricarballylate, and aluminum lactate were synthesized

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1) D. H. Copp, *et al.* : UCRL-1464(1951) (C. A., **46**, 11398(1952)).
2) M. Uchiyama : Yakugaku Zasshi, **78**, 251(1958).
3) S. Akiya, M. Uchiyama : Seikagaku, **28**, 154(1956).
4) J. Schubert, H. D. Wallace, Jr. : J. Biol. Chem., **183**, 157(1950); J. Fried, M. White Rosenthal, J. Schubert : Proc. Soc. Exptl. Biol. Med., **92**, 331(1956); Y. Ito, *et al.* : This Bulletin, **6**, 34, 92(1958).

by the same procedure as in the case of DAA, and their activities were compared in making phosphate insoluble *in vitro*. It was found that aluminum tricarballylate and aluminum lactate *in vitro* had stronger tendency than DAA to make phosphates insoluble, but aluminum citrate scarcely showed this activity.

Further, the effect of these three salts on the amount of phosphate in urine and feces of mouse after their oral administration was examined and all three salts showed the activity of decreasing the urinary excretion of phosphate. Surprisingly enough, aluminum citrate which had no activity *in vitro* had the apparent effect of making phosphates much more insoluble than DAA *in vivo*.

Moreover, the mice showed a similar appetite for the experimental diet consisting of normal food plus aluminum citrate as for normal food and the aluminum citrate-treatment did not give any untoward effect on ordinary growth of mice. The administration of other two salts, however, caused a considerable decrease in the appetite and an insufficient growth of the mice.

Although administration of aluminum tricarballylate or aluminum lactate caused decreased excretion of the phosphate not only in the urine but also in the feces, treatment with aluminum citrate, on the contrary, caused increase of phosphates in the feces proportionally with its decrease in the urine from those in the control.

From the above results, aluminum citrate was expected to possess the most efficient activity for eliminating the poisoning of radioactive strontium. The effect of this salt on excretion of the injected radioactive strontium was examined using mice and strontium-90, comparing with those of the other aluminum salts. It was observed that the efficacy of aluminum citrate against this poisoning for the duration of both 24 hours and 6 days was statistically more significant than the other salts including DAA. A simultaneous administration of calcium with aluminum citrate was also attempted, but it did not give a clear-cut result and further investigations are required on this point.

Experimental

Phosphate Insolubilization by Aluminum Compounds *in vitro*—Each solution of 0.12 mole of the acid, i.e. citric, tricarballylic, or lactic acid, in dehyd. iso-PrOH was added to 0.1 mole of Al(iso-PrO)$_3$ (20.4 g.) dissolved in 300 cc. of dehyd. iso-PrOH, under stirring. The precipitate was collected and washed repeatedly with iso-PrOH. After drying, the salt was used for the experiments.

DAA, Al(OH)$_3$, Al-tricarballylate, Al-lactate, or Al-citrate was dissolved in dil. HCl (pH 1.5) to make 5 m*M* solutions. 20 cc. of each solution was placed in a test tube with a glass stopper. After keeping in a water bath at 38°, 1 cc. of 100 m*M* solution of KH_2PO_4 was added to each test tube. When pH of these mixtures was adjusted to 6.9 with Na_2CO_3 solution, a precipitate of $AlPO_4$ appeared. Phosphates in the supernatant were determined colorimetrically following Allen's method.[5] The results are given in Fig. 1.

Phosphate Insolubilization by Aluminum Compounds *in vivo*—Five groups of mice were used in this experiment. Each group consisted of 5 mice and each group was kept in a separate cage. The bottom of the cage was arranged with water-proof steel net. On the first day, all mice were fed with 2 g. of normal diet, and the urine and feces excreted were collected on a filter paper placed under the steel net. On the second day, the mice in the control group were fed with normal diet, same as the first day, while the mice in other four groups were fed with respective diet mixed with 1 m.mole each of DAA, Al-tricarballylate, Al-lactate, or Al-citrate. Urine and feces were collected during the next 24 hr. Phosphates in urine and feces were determined by Allen's method. The results are given in Fig. 2.

Effect of Aluminum Citrate on the Elimination of Radioactive Strontium—Four groups, each of which consisted of 5 mice, were used. All mice were trained to eat the diet on the steel net. After fasting, 0.1 μc of radioactive strontium dissolved in 0.1 cc. of saline was subcutaneously injected to the mice. Each mouse was given 2 g. of normal food containing DAA, Al-tricarballylate, or Al-citrate. After 24 hr., all mice were sacrificed. Excreta and carcasses were ashed, dissolved in *N* HNO_3, and the radioactivity measured after standing for sufficient period for the radio-equili-

5) R. J. L. Allen: Biochem. J., **34**, 858(1940).

brium of ^{90}Sr—^{90}Y.

In a separate experiment, 1 m.mole of Al-citrate and 0.1 g. $CaCO_3$ were administered for 6 days and results are given in Tables I and II. Except for Al-citrate, all aluminum salts were found not suitable for such long-period treatment because they suppressed the appetite of mice.

Results and Discussion

Relative activity of each aluminum compound on phosphate insolubilization *in vitro* is shown in Fig. 1. Aluminum tricarballylate and aluminum lactate have a stronger ten-

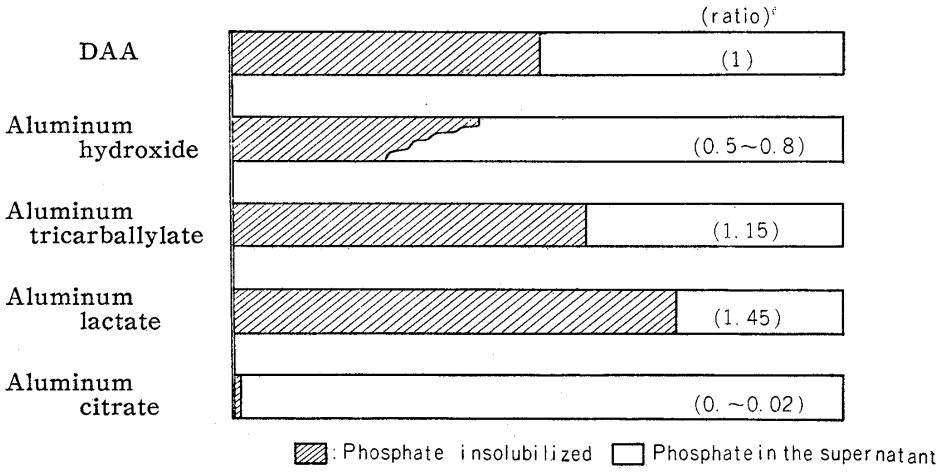

Fig. 1.
Effect of Aluminum Compounds on the Insolubilization of Phosphates *in vitro* at pH 6.9

dency for insolubilizing phosphates *in vitro* than DAA, but in the case of aluminum citrate, aluminum phosphate did not appear when pH of the reaction mixture was adjusted to 6.9. Thus, under such condition *in vitro*, aluminum citrate scarcely insolubilized the phosphates. Further, when citric acid was mixed with DAA or other aluminum compounds, these aluminum compounds lost their activity to make phosphates insoluble *in vitro*. Therefore, it can be mentioned that citrate inhibits the production of aluminum phosphate from phosphates and aluminum compounds *in vitro*.

Inhibition of gastrointestinal absorption of phosphates is shown in Fig. 2. The amount of phosphates excreted in urine decreased markedly by the administration of aluminum compounds. It is of interest that aluminum citrate, different from the result

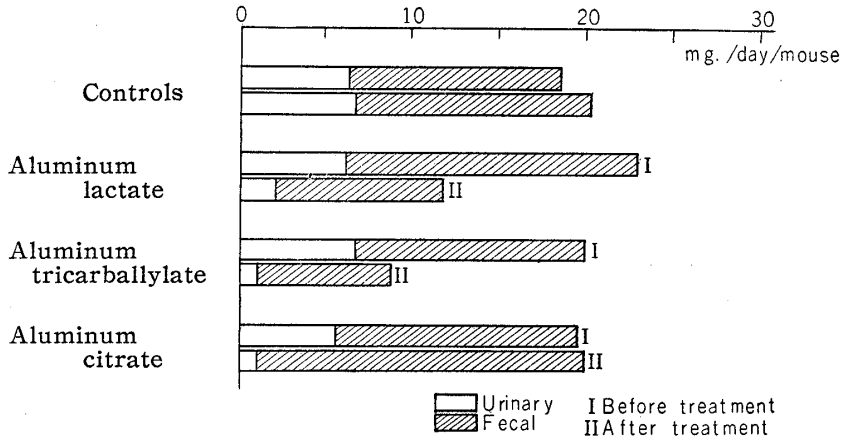

Fig. 2. Influence of Aluminum Compounds on the Amount of Urinary and Fecal Phosphates

obtained *in vitro*, appeared to make phosphates insoluble *in vivo*. If this suppression of phosphate excretion is caused by phosphate insolubilization by the agent in the gastrointestinal tract, the precipitated phosphates must be excreted in feces and the amount of phosphates in feces must increase. In the experiments for aluminum citrate, it was observed that phosphate in feces increased by the same amount as it decreased in the urine. In the case of aluminum tricarballylate and aluminum lactate, the decrease in the amount of the phosphates was observed both in urine and feces. However, because the food containing these two salts suppressed the appetite of the animals, the decrease in the total amount of phosphate excreted may have been caused by diminished in-take of the diet. For the above phosphate-insolubilizing effect of aluminum citrate, the citric acid moiety seemed not important, and sodium citrate and citric acid are known to have no inhibiting activity for the gastrointestinal absorption of phosphates.

From these facts, aluminum citrate clearly seems to make the phosphate insoluble in the gastrointestinal tract. That this activity is considered to be caused by the secondary insolubilization of phosphates is supported by the following experiments: Two dialysis sacs, containing a mixture of dil. hydrochloric acid solution of aluminum citrate and potassium dihydrogenphosphate solution adjusted to pH 6.9, were prepared. The one was immersed in distilled water and the other in sodium citrate solution, the molar concentration of which was the same as that of aluminum citrate in inner solution. After a few hours, the precipitation of aluminum phosphate appeared in the former sac, but not in the latter.

From such a result, it is considered that citrate inhibits the phosphate insolubilization by aluminum compounds, but when the citrate is removed (by dialysis *in vitro* or by absorption *in vivo*) the precipitation of aluminum phosphate occurs. The reason why aluminum citrate makes the phosphates insoluble *in vivo* but not in *in vitro* seems to be due to the simultaneous intestinal absorption of citrate.

Previously, it was found by Ito, *et al.*[6] that the effectivity of low-phosphorus diet on elimination of radioactive strontium is due to stimulation in excretion of calcium. On the other hand, investigators[7] have found that the oral administration of citric acid causes increasing excretion of urinary calcium and strontium. Although there are many opinions on the mechanism of this phenomenon, this stimulation in the urinary excretion of strontium by citrate administration is generally accepted. Therefore, the more effective elimination of radioactive strontium could be expected by the administration of aluminum citrate which is assumed to have both the activity of increasing absorption of calcium and insolubilization of phosphate in intestinal tract.

Effect of aluminum citrate for the elimination of radioactive strontium is given in Table I. All aluminum salts tested, DAA, aluminum citrate, and aluminum tricarballylate, showed considerable effect of the strontium elimination compared with the control,

Table I. Effect of Aluminum Compounds on the Elimination of Radioactive Strontium in Mice (24 hr.)

	Injected ^{90}Sr Retained (%)			
	Control	DAA (1 m*M*)	Al-tricarb. (1 m*M*)	Al-citrate (1 m*M*)
	65.1	51.9	41.4	36.4
	66.2	52.8	33.9	46.6
	58.5	47.8	33.8	42.0
	70.9	45.4	44.8	29.6
	62.6	48.6	42.8	33.4
mean ± S.E.	64.7 ± 4.1	49.3 ± 2.7	39.3 ± 4.6	37.6 ± 6.1

6) Y. Ito, S. Tsurufuji, M. Shikita, S. Ishibashi: This Bulletin, **6**, 115(1958).
7) T. S. Chang, S. Freeman: Am. J. Physiol., **160**, 330(1950).

and the activity of aluminum citrate was significantly higher than that of DAA. Mice did not like to eat the diet mixed with aluminum tricarballylate and left one-half of the food given. Thus, the body weight of the mice administered with this salt did not show any increase.

In this point, there was no other aluminum compound than aluminum citrate which could be fed in such a considerably large amount without any undesirable effect on the animals. The result of long-term administration of aluminum citrate is shown in Table II.

Table II. Effect of Aluminum Compounds on the Elimination of Radioactive Strontium in Mice (6 days)

	Injected ^{90}Sr Retained (%)		
	Control	Al-citrate	Al-citrate+$CaCO_3$
	55.5	37.5	21.2
	51.5	28.1	20.8
	62.0	31.4	43.7
	52.8	38.0	47.7
	50.8	16.7	20.7
	52.8	33.0	22.2
mean±S.E.	54.1±3.8	30.8±7.2	29.4±12.3

Expecting that the greater absorption and excretion of calcium by administration of a higher concentration of calcium could increase the elimination of strontium, calcium carbonate was mixed with aluminum citrate, but in this case no clear-cut result on strontium excretion was observed.

The results of experiments presented here reveal that, at least in present step, oral administration of aluminum citrate is the most effective for elimination of radioactive strontium from the body.

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Summary

Aluminum citrate, aluminum tricarballylate, and aluminum lactate were prepared to test their activity for insolubilization of the phosphate, both *in vitro* and *in vivo*, and for facilitating the excretion of administered radioactive strontium, in comparison with those of dihydroxyaluminum aminoacetate, which was previously reported as an effective agent for elimination of strontium.

According to the results of experiments *in vitro*, aluminum tricarballylate and aluminum lactate have a stronger tendency to make phosphate insoluble, but aluminum citrate has almost no activity. In the case of experiment *in vivo*, on the contrary, aluminum citrate showed a considerable inhibition of gastrointestinal absorption of phosphate, and moreover, it showed no untoward effect on the growth of animals, while the other two salts seem to reduce their appetite.

The effect of aluminum citrate on the elimination of strontium is shown in Tables I and II. By aluminum citrate treatment, 60% of administered ^{90}Sr was excreted in 24 hours and 70% in 6 days, compared with respective 35% and 45% in control animals.

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