Note : For spray reagent Horrock's benzidine reagent is used.

1) These spots are suggested to correspond to gentiobiose which is not tested.

2), 3), 4), and 5) These spots are never recorded but these are supposed to be polymers of sugar.

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15. Studies on the Urinary Phosphatase

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Man's urine (4 l.) was adjusted to pH 4 with acetic acid. The urinary enzyme was absorbed with 100 g. of *bolus alba* (or kaolin) and eluted at pH 8. After dialysing at pH 4, it was precipitated with $(NH_4)_2SO_4$ of 60 % saturation. The precipitated enzyme was dialysed and used in the experiments (15 ml.). It was regarded as the prostatic enzyme (Kutscher *et al.*, *Z. physiol. Chem.* **236**, 237 (1953) ; Abul-Fadl., *Biochem. J.* **45**, 51 (1949)). A mixture of 2 % β-glycerophosphate, acetate buffer solution and enzyme was incubated at pH 5.5 and 37°C and then the free P was measured by Allen's method. In order to distinguish the urinary phosphatase from the acidic kidney phosphatase the test mixtures were digested at 37°C for 60 mins. with EtOH or AcOAc. The results showed that the former was more resistant against HCHO and more sensible to EtOH andAcOAc, while the latter was inactivated by HCHO and not influenced by AcOAc and EtOH. To confirm the effects of pH upon the enzymes, the acetate buffer of several pH values was added to the enzyme solution and then the enzyme-buffer solutions were kept at 37°C for 90 mins.; after adjusting pH to 5.5 they were mixed with substrate and digested in order to examine the enzyme activities. It was confirmed that the enzyme activities were not decreased at all in pH range from 3 to 7, but at the higher pH value, their activities were lost. So the enzyme appeared to be very stable at the acid condition. In a centrifuge tube, precipitation of the urinary enzyme by acetone was performed at several pH ranges. 0.2 ml. of enzyme, 0.2 ml. of acetate-buffer and 5.0 ml. of cold AcOAc were mixed, and the mixture was kept at 0°C for 30 mins. and on centrifuging the supernatant was removed; to the precipitant, 1 ml. of acetate-buffer of pH 5.5 and 0.2 ml. of 2% β-glycerophosphate solution were added and digested under toluene to measure the activity of the enzyme. At pH 7 its activity was observed to remain sufficiently well, but it was decreased suddenly by the acid treatment and greatly damaged below pH 4 (Fig. 1). To ascertain the activity of the supernatant, it was aerated to evaporate AcOAc, the residual matter was dissolved in a buffer of pH 5.5 and its enzymatic activity was measured. No activity was observed in the acid side supernatants,

(Hydrolytic value without AcOAc is regarded as 100 %)

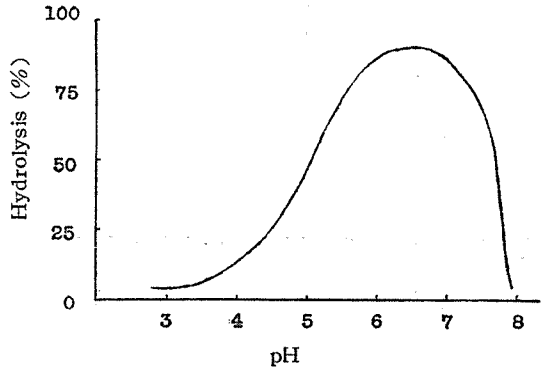

Fig. 1 Hydrolysis of β-glycerophosphate by urinal phosphatase precipitated by AcOAc at several pH values.

and only a little in the alkaline side supernatants, which gave slight acetone precipitation. As mentioned above, the inactivation of the urinary phosphatase by acetone was odserved to be affected by the pH of medium and at the neutral it could not be resulted in a short time. In 1936 Kutscher and Wörner reported on the irreversibility of AcOAc inactivation of the urinary phosphatase at pH 5.4 (*Z. physiol. Chem.*, **239**, 109) which was assured here by us as follows: A mixture of 0.2 ml. of *N*/50HCl, 0.2 ml. of enzyme solution and 5.0 ml. of AcOAc (pH 5.5) was kept at room temperature for 30 mins. and it was centrifuged to separate the enzyme from the supernatant. AcOAc was evaporated from the supernatant and the pH of the residual liquid was made 7.0 by *N*/50 NaOH ; then it was added to the precipitated enzyme, but no activity seemed to be recovered. In 1949 Abul-Fadl found that the inactivation by L-tartrate was recovered by dialysis. So we have dialysed the enzyme solution inactivated by acid acetone treatment, but no activity was recovered. However, when a fresh urine was added to the acid acetone inactivated enzyme, a remarkable activity was found in a short time. Even if we have heated the inactivated enzyme at 100°C for 3 mins., the phosphatase value was not decreased after urine addition. But when the fresh urine was heated, the activating effect was not recognized.

Table 1. The phosphatase activity of a mixture of the enzyme inactivated by acid-acetone treatmemt and a fresh urine. (pH 5.5, 37°C, 30 mins.)

	Inorganic P value increased (γ in 1.7 ml.)				
Fresh urine (control 1)	30	137	10	192	190
Inactivated enzyme (control 2)	10	14	0	3	7
Inactivated enzyme + fresh urine	71	194	23	249	251
Inactivated eezyme + heated fresh urine	—	—	3	—	8
Heated inactivated enzyme + fresh urine	—	—	19	249	—

Test mixture contains inactive enzyme precipitated by AcOAc, 0.5 ml. fresh urine, 0.2 ml. 2 % β-glycerophosphate and 1.0 ml. buffer of pH 5.5.

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16. Studies on the Components of Japanese Toxic Plants. (I)

On the Toxic Components of *Coriaria Japonica*

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We have isolated from the leaves of *Coriaria japonica* indigeneous to Japan "coriamyrtin" $C_{15}H_{18}O_5$, and "tutin" $C_{15}H_{18}O_6$, and found that they resemble picro toxinin, a neurotropic poison obtained from *Anamirta paniculata* of India, in the chemical properties and physiological actions.

Coriamyrtin was aromatized on boiling with HI and red phosphorus to coriaria-lactone $C_{15}H_{18}O_2$ (I) which may be considered to retain almost all features of the coriamyrtin skeleton. Oxidation of coriaria-lactone by alkaline solution of permanganate yielded a dicarboxylic acid, coriaric acid $C_{15}H_{16}O_6$ (II), which, on further oxidation with acid solution of permanganate, gave a dilactone, coriaria-dilactone $C_{14}H_{14}O_4$ (III), by decarboxylation of the tertiary carboxylic acid. By the action of CH_3MgI on benzene-1,2,3,4-tetracarboxylic acid dianhydride coriaria-dilactone was synthesized which showed no depression of mp. with (III), and both produced acetone and phthalic acid by alkali fusion. The mechanism of the reaction from coriaria-lactone to coriaria-dilactone *via* coriaric acid was ascertained by a model reaction from dimethyl hydrinden to dimethyl phthalide. It was ascertained from the results of experiments that tertiary carboxylic acids having benzene nucleus in the α-position are generally oxidized by acid solution of permanganate with evolution of carbon dioxide and produce the corresponding tertiary alcohols.

It has been reported by Mercer, Robertson *et. al.* that picrotoxinin is aromatized by HI and red phosphorus to picrotic acid (*J. Chem. Soc.* **1935**, 997), which has similar structure to coriaria-lactone.

Besides the similarity of the structures of aromatized derivatives we found that coriamyrtin has several common chemical properties with picrotoxinin. It was known that they have one hydroxyl group from Zerevitinov determination, and one $>C=CH_2$ group from the combined results of bromination, hydrogenation, and ozonization. They produce monobromo-substituted product when bromine is added to their hot aqeous solution. It may be suspected from ester number that coriamyrtin has one lactone group and picrotoxinin two lactone groups. They reduce Fehling's solution