N-(1,4-Dihydro-3-ethoxycarbonyl-1-ethyl-4-oxo-1,8-naphthyridine-7-yl)methylenamines (4—15)——General Procedure: A mixture of **2** (0.001 mole) and respective amines (0.001 mole) in EtOH (10 ml) was heated at the reflux for 1—3 hr. The precipitated solid was filtered and recrystallized from proper solvent listed in Table I to give the corresponding product. When the product did not precipitated out from the cooled reaction mixture, the solution was evaporated to dryness *in vacuo* and the residue was covered with ether to separate the product.

In the case of **4**, **12**, **13**, and **15**, the corresponding amine–HCl was used. In these cases, the amine–HCl was dissolved in H_2O (0.5 ml) and heated with **2** in EtOH.

N-(3-Carboxy-1,4-dihydro-1-ethyl-4-oxo-1,8-naphthyridin-7-yl)methylenamines (16—27)——General Procedure: A mixture of **3** (0.001 mole) and respective amines (0.001 mole) in EtOH (10 ml) was heated at the reflux for 1—3 hr. The precipitated solid was filtered, and recrystallized from proper solvent listed in Table I to give the corresponding product.

In the case of **16**, **24**, **25**, and **27**, the corresponding amine-HCl was used. In these cases, the amine-HCl was dissolved in H_2O (0.5 ml) and heated with **3** in EtOH.

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Inhibition of Alkaline Phosphatase from Human Placenta and Intestine by Inorganic Phosphate[1]

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The degree of the inhibition of alkaline phosphatases from human placenta and intestine by inorganic phosphate was examined and its results showed an additional difference in alkaline phosphatases between human placenta and intestine in the inhibition by inorganic phosphate.

Keywords——human alkaline phosphatases; serum alkaline phosphatase; placenta; intestine; inhibition by inorganic phosphate

It is known that alkaline phosphatase (E. C. 3.1.3.1) is inhibited by inorganic phosphate. This fact shows that a serine residue of alkaline phosphatase is phosphorylated and its dephosphorylation process is the rate-determining step, and the phosphorylated enzyme is stable in an acidic region.[3] For this reason, the relationship between alkaline phosphatase and inorganic phosphate seems very important.

In a previous paper,[4] we reported the comparison of properties of the purified alkaline phosphatase from human placenta and intestine, and there was a difference in their sensitivity to inorganic phosphate.

In the present work, the degree of inhibition of purified alkaline phosphatases from human placenta and intestine, and human serum alkaline phosphatase by inorganic phosphate were examined.

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3) M. Fosset, D. Chappelet-Torde, and M. Lazdunski, *Biochemistry*, **13**, 1783 (1974).
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Materials and Methods

Assay of Alkaline Phosphatase——Phenyl phosphate was used as a substrate.[5] A mixture of 2 ml of 5 mM substrate in 0.1 M carbonate buffer (pH 10.5) containing 2 mM 4-aminoantipyrine was preincubated at 37°, and 0.1 ml of the enzyme solution was added. The enzyme reaction was stopped by adding 2 ml of 0.2 M boric acid solution containing 6 mM potassium fericyanide and absorbancy at 500 nm was determined. Potassium phosphate was used as the inorganic phosphate and dissolved in 0.1 M carbonate buffer. Inhibition by inorganic phosphate was determined in the same way as above for alkaline phosphatase assay.

Purified Human Placental Alkaline Phosphatase——Crude preparation of the enzyme was obtained from the fresh human placenta by the Morton method.[6] It was purified by ammonium sulfate precipitation and passed through columns of diethylaminoethyl (DEAE)–cellulose, Sephadex G-150, and carboxymethyl (CM)–cellulose, as described previously.[7] The disc electrophoresis revealed a single protein band stainable with Amido Black 10B on polyacrylamide gel.

Purified Human Intestinal Alkaline Phosphatase——Human intestinal alkaline phosphatase was extracted from human intestinal mucosa by the modified Morton's butanol method[6] and was purified by ammonium sulfate precipitation followed by passing through columns of DEAE–cellulose, CM–cellulose, and Sephadex G-200 as described previously.[8] The purified enzyme exhibited a single protein band on polyacrylamide gel in disc electrophoresis.

Human Serum Alkaline Phosphatase——The serum mainly containing the intestinal or placental alkaline phosphatase was used as the specimen.

Results and Discussion

As shown in Fig. 1, placental and intestinal alkaline phosphatases were inhibited by inorganic phosphate. The magnitude of inhibition by inorganic phosphate differed for each enzyme and the inhibition of placental alkaline phosphatase was greater than that of intestinal alkaline phosphatase at each concentration of inorganic phosphate. Serum alkaline phosphatase exhibited the same behaviors as purified alkaline phosphatase against inorganic phosphate.

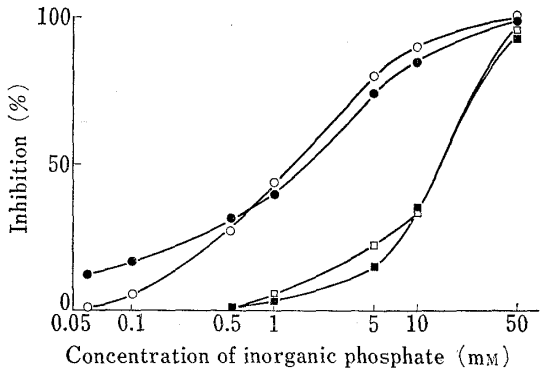

Fig. 1. Inhibition of Alkaline Phosphatase by Various Concentrations of Inorganic Phosphate

Inhibition was expressed as the percentage of control.
—○—: purified human placental alkaline phosphatase
—●—: human serum rich in placental alkaline phosphatase
—□—: purified human intestinal alkaline phosphatase
—■—: human serum rich in inteetinal alkaline phosphatase

Inhibition of purified alkaline phosphatases by inorganic phosphate was competitive, while the serum alkaline phosphatase exhibited a mixed inhibition type against inorganic phosphate, probably because the serum used here contained some other organ-specific alkaline phosphatases. Concentrations of inorganic phosphate exhibiting 50% inhibition of placental and intestinal alkaline phosphatase were 1.5 mM and 15 mM in the reaction system, respectively.

In clinics, serum alkaline phosphatase is distinguished by heat stability,[9] urea,[10] and inhibition of L-phenylalanine[11] and L-homoarginine.[12] However, properties of placental and intestinal alkaline phosphatases were very similar, except for heat stability.[4] The

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present results show an additional difference between the alkaline phosphatase of human placenta and intestine in the inhibition by inorganic phosphate, which fact further emphasizes their distinctiveness and suggests that this property can be used to distinguish the original tissue of the alkaline phosphatases in serum in combination with L-phenylalanine inhibition, without the use of heat stability method.

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Bromination of 3-*tert*-Butylindoles with N-Bromosuccinimide

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Reaction of 3-*tert*-butylindole (**1a**) with NBS in acetic acid gave 3-*tert*-butyloxindole (**2a**) and 6-bromo-3-*tert*-butylindole (**3a**) instead of 2-bromo derivative (**5a**). Reactions of 1-acetyl-(**1b**) and 1-*tert*-butyl-(**1c**) derivatives under similar conditions gave 3-bromo-oxindole (**6b**) and the brominated indole (**7**) respectively. On the other hand reaction of **1a** with NBS in boiling carbon tetrachloride in the presence of benzoyl peroxide gave unstable **5a** as a main product. Reaction of **1b** under similar condition did not proceed, but **1c** gave **7** as a main product.

Keywords——bromination; 3-substituted indoles; N-bromosuccinimide; solvent effect; 6-bromoindoles; 2-bromoindoles; 3-substituted oxindoles

We have previously reported the bromination of 3-phenylindoles[2] and 3-methylindoles[3] with N-bromosuccinimide (NBS) in acetic acid or in carbon tetrachloride. This paper describes the reaction of 3-*tert*-butylindoles (**1**) having a bulky substituent at 3-position with NBS in acetic acid or carbon tetrachloride.

The reaction of 3-*tert*-butylindole (**1a**) with NBS in acetic acid at 20° gave 3-*tert*-butyloxindole (**2a**, 24%), mp 161—162°, 6-bromo-3-*tert*-butylindole (**3a**, 22%), mp 89.5—91.0°, and brominated 3-*tert*-butyloxindole (**4a**, 0.8%) along with the recovered starting material (47%). The corresponding 2-bromo derivative (**5a**) was not isolated in contrast with the bromination of 3-phenyl- and 3-methylindoles,[2,3] but the 6-brominated derivative (**3a**) without affecting at 2-position was isolated for the first time. The position of bromine atom in **3a** was confirmed by the nuclear magnetic resonance (NMR) spectrum of its 1-acetyl derivative (**3b**) which showed a down field fine doublet for 7-H.

The reaction of 1-acetyl-3-*tert*-butylindole (**1b**) under the same condition gave 1-acetyl-3-bromo-3-*tert*-butyloxindole (**6b**, 28%), mp 82.5—83.5°, and the 6-bromoindole (**3b**, 4%) besides the recovered **1b** (58%). 3-Bromo-oxindole derivatives have been obtained by the bromination of 1-acetyl-3-phenyl- and 3-methylindoles.[2,3]

The reaction of 1,3-di-*tert*-butylindole (**1c**) which was obtained as a by-product in the preparation of 3-*tert*-butylindole, with NBS under the same condition gave the brominated 1,3-di-*tert*-butylindole (**7**, 30%), the oxindole (**2c**, 5%), and 3-bromo-oxindole (**6c**, 5.5%) along with

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