The method for determination of creatinine with DNSK is comparatively simple and rapid one as shown in the experiments described above. Moreover, the recoveries of urinary creatinine gave more accurate results with DNSK than with picric acid, and standard error of the value was very small.

Acetone, which was supposed to increase the color density and be contained in normal urine, did not affect the estimation of creatinine. In the presence of a large amount of acetone, 160 γ per ml., slight increase of the recovery value (about 4%) was observed. However, such a large amount of acetone was not excreted in usual cases.

The authors express their gratitude to Dr. Akatsuka for supplying 3,5-dinitrobenzenesulfonate.

Summary

For the estimation of urinary creatinine, DNSK-reagent was used and the estimation was satisfactorily carried out under the conditions as follows;

1) Sample urine is prepared by diluting with water to contain 10~80 γ per ml. of creatinine.

2) To 4 ml. of sample urine, each 0.5 ml. of 2% DNSK-reagent and 5% KOH solution is added, and after 25 minutes the color developed is estimated at 530 mμ.

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109. Yuichi Kanaoka, Osamu Yonemitsu, Kazutaka Tanizawa, and Yoshio Ban: Polyphosphate Esters as Synthetic Reagent. I.*[1] Synthesis of 2-Substituted Benzimidazoles.

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Research on phosphoric acid derivatives, covering an extremely wide spectrum in type and complexity, has been the subject of considerable effort.[1] The dramatic advances in the field of intermediary metabolism have been dependent upon the development of phosphorus chemistry.[2,3] In regard to organic chemical side, many effective synthetic agents have arisen based on this background. Polyphosphoric acid (PPA) may be a typical instance and its utility as an agent for acid-catalysed reactions, particularly for many condensation reactions, has been fully established.[4,5] In this laboratory, attempts have been made to find synthetic tools in phosphate ester derivatives. This first paper describes the application of "polyphosphate ester" or "metaphosphate" as reagent for benzimidazole synthesis.

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2) H.G. Khorana: "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest," (1961), John Wiley & Sons, Inc., N.Y.
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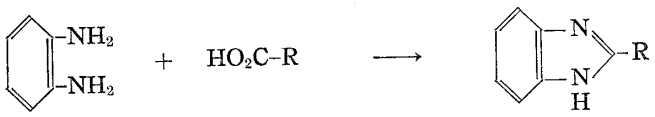
5) F. Popp, W.E. McEwen: Chem. Revs., 58, 321 (1958).

In 1910 "Metaphosphorsäureäthylester" was described by Langheld, who prepared this compound by reaction of diethyl ether with phosphorus pentoxide, and suggested even some preliminary trials as a condensing agent.[6] His early study was followed by many investigations of other groups.[7~10]

This "Langheld-Ester," initially assumed to be the monomer, is now believed to be polymeric in its nature.[11,12] Although it has a powerful phosphorylating activity, as well as condensing power as Schramm extensively claimed in his recent synthesis of polypeptides, polysaccharides, and polynucleotides,[13] its limitation as phosphorylating agent was pointed out.[14,15] Nevertheless, the use of this mixture *in situ* as a synthetic tool seems still of value, as now being reported.



I II IIIa~i

In the course of our studies on peptides, the reaction of carboxylic acids with *o*-phenylenediamine was explored in connection with C-terminal chemistry. *o*-Phenylenediamine (I) reacts readily with many carboxylic acids (II) to give 2-substituted benzimidazoles (III). The important modification, referred to as Phillips' method, involves heating II with I in aqueous hydrochloric acid.[16] In general aliphatic acids afford good yields of III, whereas with aromatic acids the yields are frequently poor.[17,18] High yields of 2-arylbenzimidazole are realized when a reaction mixture is heated at 180° in a sealed tube.[19,20] The use of PPA instead of mineral acid gave a good result and this method was employed for synthesis of 2-substituted benzimidazoles[21] and dibenzimidazoles.[22] Although these modifications have opened a good scope, the reaction conditions of Phillips' synthesis, including any previously described improved methods, seem too drastic for application to compounds which contain various groups susceptible to side reactions. Since peptides possess many peptide bonds and other sensitive groups, it is obvious that Phillips' synthesis, involving mineral acid treatment while heating, is not suitable with peptide as substrate. Even PPA procedure requires elevated temperature (250°) and long heating time (2~4 hr.),[21] and may cause undesirable confusion.

Polyphosphate ester (herein abbreviated as PPE)*3 was found to be a highly effective and convenient reagent for this condensation and demonstrated to be a general reagent for Phillips' benzimidazole synthesis.

6) *a*) K. Langheld: Ber., **43**, 1857 (1910); *b*) *Ibid.*, **44**, 2076 (1911).
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9) W. Stecker, H. Heuser: Chem. Ber., **57**, 1364 (1951).
10) R.H.A. Plimmer, W.J.N. Burch: J. Chem. Soc., **1929**, 292.
11) R. Rätz, E. Thilo: Ann., **572**, 173 (1951).
12) F. Cramer, H. Hettler: Chem. Ber., **91**, 1181 (1958).
13) G. Schramm, H. Grötsch, W. Pollmann: Ang. Chem. Intern. Edn., **1**, 1 (1962).
14) ref. 2), p. 22.
15) ref. 3), p. 108.
16) *a*) M.A. Phillips: J. Chem. Soc., **1928**, 172; *b*) *Ibid.*, **1928**, 2393; *c*) *Ibid.*, **1929**, 2820; *d*) *Ibid.*, **1931**, 1143, and other papers.
17) J.B. Wright: Chem. Revs., **48**, 397 (1951).
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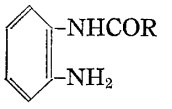
21) D.W. Hein, R.J. Alheim, J.J. Leavitt: J. Am. Chem. Soc., **79**, 427 (1957).
22) L.L.Y. Wang, M.M. Joullie: *Ibid.*, **79**, 5706 (1957).



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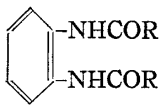

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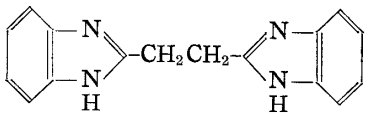


Ⅵ

By the use of excess of PPE both as a condensing agent and reaction media, aliphatic acid, such as acetic or propionic acid, gave corresponding 2-alkylbenzimidazole (Ⅲa or Ⅲb) by heating at 100° for 10 minutes in fairly good yield. Benzoic acid gave, however, no benzimidazole under the above conditions, mono-(Ⅳ : R=C_6H_5) and/or diamide (Ⅴ : R=C_6H_5) being only product isolated. By heating at 120° for 20~40 minutes, 2-phenylbenzimidazole (Ⅲc) was obtained. Relation between reaction conditions and yields for aliphatic and aromatic acids is shown in Fig. 1. It was reported that aromatic acids containing nitro groups led to carbonization because of the oxidative effect of the nitro group at a high temperature employed in usual condition.[23] With PPE as an agent, however, 2-(*p*-nitrophenyl)benzimidazole (Ⅲd) was easily obtained.

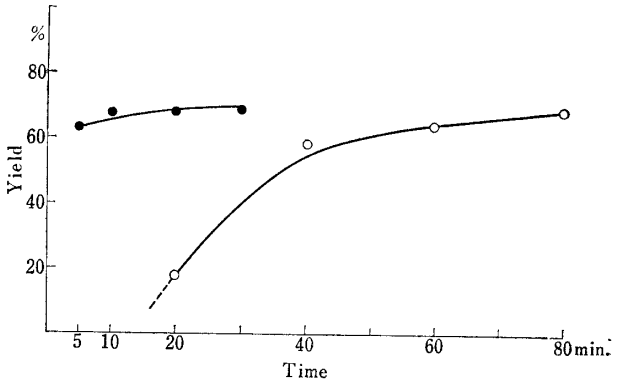


Fig. 1. Relation between Time and Yield

Acid (5 mmoles) was reacted with I (7.5 mmoles) in the presence of PPE as described in the general procedure.

—●—●— Ⅲa, at 100°
—○—○— Ⅲc, at 120°
------ amide formation

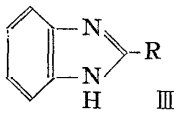
Hughes and Lions prepared 2-benzamidomethylbenzimidazole (Ⅲh) by fusion of hippuric acid and I.[24] Ⅲh and 2-(1-benzamidoethyl) benzimidazole (Ⅲi) were prepared by fusion or heating with acid media in a sealed tube.[25] Recently, Phillips' method was shown to be successful for α-amino acids only if the refluxing period is extended to more than 30 hours.[26] By PPE method, hippuric acid and N-benzoyl-DL-alanine gave corresponding benzimidazoles (Ⅲh, i) at 100° in 10 minutes in a moderate yield. This finding of extremely mild conditions for acylamino acids led us to hope that this modification of Phillips' synthesis would be applicable to peptide chemistry as a novel C-terminal determination method.

Employment of strong acid under conventional conditions made it difficult to be applied for acid-sensitive heterocyclic compounds. PPE method is again useful for synthesis of the heterocyclic derivatives of Ⅲ starting from corresponding heterocyclic acids. For illustration, 2-(2-furyl)benzimidazole (Ⅲg), which had been prepared only by Weidenhagen's aldehyde procedure from furfural,[27] was readily prepared by PPE method. 2-Substituted benzimidazoles prepared in this manner are listed in the Table I.

PPE method was also effective for the condensation of anhydride. By the reaction with I, acetic anhydride gave 2-methylbenzimidazole (Ⅲa). Succinic acid, an example of dibasic acid, gave 2,2'-ethylenedibenzimidazole (Ⅵ) as expected.

*3 As discussed in this paper, there is little doubt in that the principal species in the reaction mixture of diethyl ether and phosphorus pentoxide in chloroform is ethyl polymetaphosphate. In this work, the nomenclature "PPE" is used for convenience simply to represent the reagent as the mixture *in situ* prepared by this procedure.

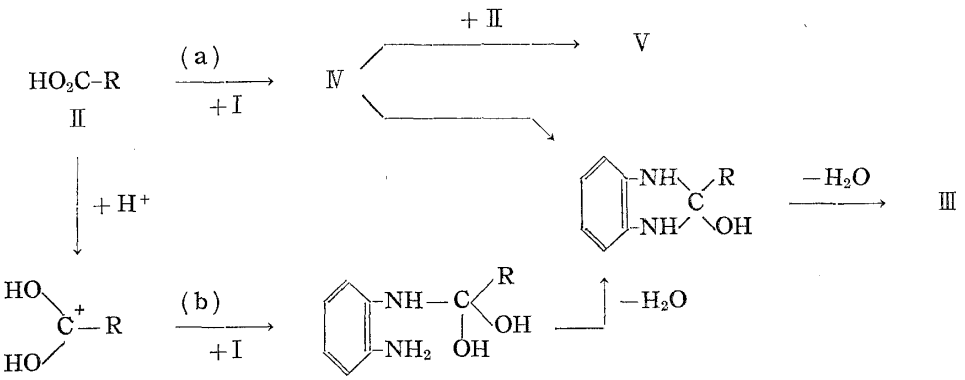
23) R. Walther, T. von Pulawski : J. prakt. Chem., [2] **59**, 249 (1899).
24) G.K. Hughes, F. Lions : J. Pro. Soc. N.S. Wales, **71**, 209 (1938) (C.A., **32**, 5830 (1938)).
25) R. Crawford, J.T. Edward : J. Chem. Soc., **1956**, 673.
26) L.A. Cescon, A.R. Day : J. Org. Chem., **27**, 581 (1962).
27) R. Weidenhagen : Ber., **69**, 2263 (1936).

TABLE I. 2-Substituted Benzimidazoles  IIIa~i

No.	Acid	Temp. (°C)	Time (min.)	Product (R)	Yield[j]	m.p. (°C)
IIIa	acetic acid	100	10	CH_3	68[l]	colorless prisms (H),[k] 174~175[a]
IIIb	propionic acid	100	10	C_2H_5	76	colorless leaflets (H), 172~173[b]
IIIc	benzoic acid	120	40	C_6H_5	58	colorless grains (A-H), 278~279[c]
IIId	*p*-nitrobenzoic acid	150	20	*p*-nitrophenyl	46	yellow powders (A), 307~310[d]
IIIe	phenylacetic acid	120	10	benzyl	51	colorless powders (A-H), 182~184[e]
IIIf	nicotinic acid	120	60	3-pyridyl	51	colorless fine needles (H), 253~254[f]
IIIg	2-furoic acid	150	20	2-furyl	68	faint brown needles (A-H), 278~279[g]
IIIh	hippuric acid	100	10	benzamidomethyl	66	colorless grains (A), 226~228[h]
IIIi	N-benzoyl-DL-alanine	100	10	1-benzamidoethyl	49	colorless powders (E), 253~255[i]

a) lit.,[16a] 178.5~179°. *b*) lit.,[16c] 177°. *c*) lit.,[16b] 290°. *d*) lit.,[28] 329~330°. *e*) lit.,[19] 189°. *f*) lit.,[21] 253.6~254°. *g*) lit.,[27] 286~290°. *h*) lit.,[24] 228°. *i*) lit.,[25] 254°. *j*) Yields of recrystallized product. *k*) Recrystn. solvent: H, H_2O; A, EtOH; E, EtOAc. *l*) The yield calculated based on UV spectrum intensity was 87%.

The mechanism of Phillips' method, together with the role of hydrochloric acid involved,[19] have been studied by many groups. The reaction was considered to proceed according to the course-(a) or (b) shown below.[28]

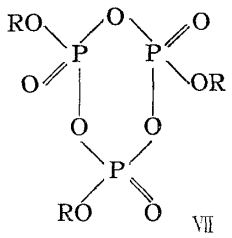


On treatment with PPE, when a reaction condition was not strong enough, corresponding mono and/or di-amide were isolated in several cases (R=C_6H_5, 3-pyridyl, and 2-furyl). Additional treatment of monoamide (IV : R=C_6H_5) with PPE effected smooth cyclization to give the final product. This result may be an illustration of the isolation of intermediate compound (IV) in the course-(a), achieved in the aprotic medium of PPE. This has hardly been realized since under the usual hydrolytic medium of Phillips' synthesis no intermediate normally survive. The occasional isolation of diamide may imply that benzimidazole cyclization requires stronger reaction conditions than amide

28) ref. 17), p. 406~408.

formation, and the latter can take place probably more easily than the former in general. Under milder conditions, therefore, the intermediate amide (Ⅳ) may undergo the second amidation with an additional mole of Ⅱ instead of the intramolecular benzimidazole cyclization thus deviating from the course-(a) to give diamide (Ⅴ) as by-product.

In view of the preparation procedure, PPE used in this work is considered to be a mixture of polyphosphate esters. Cramer reported that the previously described "Metaphosphorsäureester" was essentially cyclic trimetaphosphate (Ⅶ).[12] Thilo also discussed the nature of the metaphosphate in the hydrolytic study of "Langheld-Ester."[11] Because of its nature of mixture, criticisms were made as a phosphorylating agent for PPE, despite of Schramm's claim. It must be noted, however, that the metaphosphate or PPE is of great historical significance since the currently used phosphorylation methods are thought to involve a metaphosphate type of intermediates.[14] In his mechanistic study of internucleotide bond synthesis by the carbodiimide method, Khorana concluded that the initial phosphorylating species is the alkyl trimetaphosphate (Ⅶ).[29] In the light of above literatures as well as the fact that the infrared spectrum of our routine PPE sample is identical with that of ethyl trimetaphosphate prepared from authentic silver trimetaphosphate and ethyl iodide by Cramer,[12] it may be a reasonable postulate that the active species of PPE is ethyl trimetaphosphate (Ⅶ : R=C_2H_5).[12] In the benzimidazole synthesis, this active species may activate probably both of carboxylic acid and *o*-phenylenediamine to initially form amide bond (Ⅳ) then cyclize to give benzimidazole. Studies on amide formation in the presence of PPE and other applications as synthetic agent will be reported elsewhere.



Ⅶ

Experimental*4

Polyphosphate Ester (PPE)——Polyphosphate ester was prepared essentially following Schramm's description.[13] The whole mixture was refluxed for 30~40 hr. The solution was decanted from undissolved material and evaporated *in vacuo* avoiding moisture to leave slightly brown viscous syrup. The IR spectrum of this sample was identical with that of ethyl trimetaphosphate prepared from silver trimetaphosphate and EtI.[12]

General Procedure for Synthesis of 2-Substituted Benzimidazoles (IIIa~i) from Aliphatic, Aromatic and Heterocyclic Acids——A mixture of *o*-phenylenediamine (810 mg.; 7.5 mmoles) and PPE (5 g.) was heated at 100~120° under mechanical stirring. After *o*-phenylenediamine went into solution, carboxylic acid (5 mmoles) was added and heating was continued for 10~20 min. under stirring. The whole reaction mixture was cooled to room temperature, H_2O (ca. 50 ml.) was added to decompose PPE. Excess of powdered $NaHCO_3$ was added to around pH 8 and then crude benzimidazole precipitated. Recrystallized usually from aq. EtOH. Results are listed in Table I. In some cases stronger conditions were required as shown.

2-Methylbenzimidazole (IIIa) from Acetic Anhydride——A mixture of I (15 mmoles) and PPE (8 g.) was heated at 100° under mechanical stirring. Acetic anhydride (5 mmoles) was added and kept at 100° for 10 min. under stirring. Working up as above, giving Ⅲa in 49% yield.

2,2'-Ethylenedibenzimidazole (VI)——Succinic acid (5 mmoles) was reacted with I (15 mmoles) at 120° for 20 min. and worked up as above. Colorless needless of m.p. 315~325° (decomp.) from EtOH (lit.,[26] 325~330° (decomp.)). Hydrochloride, UV λ_{max}^{EtOH} mμ (log ε) : 271.5 (4.34); 276.5 (4.39).

Isolation of Amide as an Intermediate——i) When BzOH (5 mmoles) was reacted with I (7.5 mmoles) (a) at 100° for 10 min., (b) at 100° for 20 min., or (c) at 120° for 10 min., a mixture of 2'-aminobenzanilide and 2'-benzamidobenzanilide were obtained in following respective amount : (a), 175 mg., 175 mg.; (b), 25 mg., 250 mg.; (c), 0 mg., 250 mg. 2'-Aminobenzanilide (Ⅳ : R=Ph) : Colorless needles of m.p. 143° from aq. EtOH (lit.,[31] 140°). 2'-Benzamidobenzanilide (Ⅴ; R=Ph) : Colorless needles of m.p.

*4 Melting points are uncorrected.

29) G. Weinmann, H. G. Khorana : J. Am. Chem. Soc., **84**, 4329 (1962).
30) R. L. Schriner, R. W. Upson : *Ibid.*, **63**, 2277 (1941).
31) A. Bistrazycke, F. Ulffers : Ber., **23**, 1878 (1890).

299~301° from DMF (lit.,[32] 300°). IR : ν_{max}^{Nujol} 1660 cm^{-1} (s) (C=O of amide). This material was identical with the authentic sample prepared from I and BzCl.

ii) **2′-Nicotinamidonicotinanilide (V : R=3-pyridyl)**——When nicotinic acid (5 mmoles) was reacted with I (7.5 mmoles) at 100° for 10 min., no benzimidazole was obtained and the diamide (150 mg.) was isolated as colorless needles of m.p. 253~254° (decomp.) from aq. EtOH. IR : ν_{max}^{Nujol} 1670 cm^{-1} (s) (C=O of amide). *Anal.* Calcd. for $C_{18}H_{14}O_2N_4$ (V : R=3-pyridyl) : C, 67.91; H, 4.43; N, 17.63. Found : C, 67.88; H, 4.54; N, 17.99.

iii) **2′-(2-Furamido)-2-furanilide (V : R=2-furyl)**——The amide (82 mg.) was obtained as above. Colorless needles of m.p. 216~217° from aq. EtOH. IR : ν_{max}^{Nujol} 1665 cm^{-1} (s) (C=O of amide). *Anal.* Calcd. for $C_{16}H_{12}O_4N_2$ (V : R=2-furyl) : C, 64.86; H, 4.08. Found : C, 64.97; H, 4.48.

Benzimidazole Formation from an Intermediate Amide in the Presence of Polyphosphate Ester——2′-Aminobenzanilide (1 part) was heated with PPE (3 parts) at 120° for 20 min. to give IIIc in 79% yield.

Ultraviolet Spectra of 2-Substituted Benzimidazoles——UV λ_{max}^{EtOH} mμ (log ε) : (IIIa), 274 (3.83), 281 (3.88); (IIIb), 274 (3.85), 281 (3.90); (IIIc), 304 (4.41); (IIId), 340 (4.31); (IIIe), 276 (3.89), 281.5 (3.93); (IIIf), 310 (4.34); (IIIg), 307 (4.45), 322 (4.34); (IIIh), 275 (3.94), 281.5 (3.94); (IIIi), 275 (3.93), 282 (3.92).

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Summary

Polyphosphate ester (PPE, or ethyl metaphosphate) was proposed as a synthetic reagent in Phillips' benzimidazole synthesis. By the use of PPE, 2-substituted benzimidazoles were prepared in very mild conditions. Literatures on the polyphosphate ester were briefly reviewed and its constitution was discussed.

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32) O. Hinsberg, L. v. Udranszky : Ann., 254, 254 (1889).