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Junichi Hase, Kyoichi Kobashi, and Kenji Kumaki : Borate Complex of Hydroxamic Acids.*1

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The inhibition of urease activity (urea amidohydrolase, EC 3.5.1.4.) by benzohydroxamic acid (I) was completely reversed by sodium borohydride (II). It was assumed that hydroxamic acids might be reduced by II to corresponding and non-inhibitory acid amides.[1] Hydroxamic acids, however, were found not to be reduced by II under the physiological condition. Therefore, the reversal of the inhibition seems to be attributed to the effect of boric acid, a decomposed product of II in the aqueous solution. In fact, by the addition of boric acid, inhibition by aromatic hydroxamic acids was completely reversed and that by aliphatic ones was completely prevented.[1] These observations suggest that hydroxamic acids, in general, form non-inhibitory complexes with boric acid under physiological condition. As is well known in the field of carbohydrate chemistry, boric acid combines with many 1,2-dihydroxy-compounds in aqueous solution to form dissociable cyclic complexes.[2] Green[3] speculated the formation of the same molar ratio com-

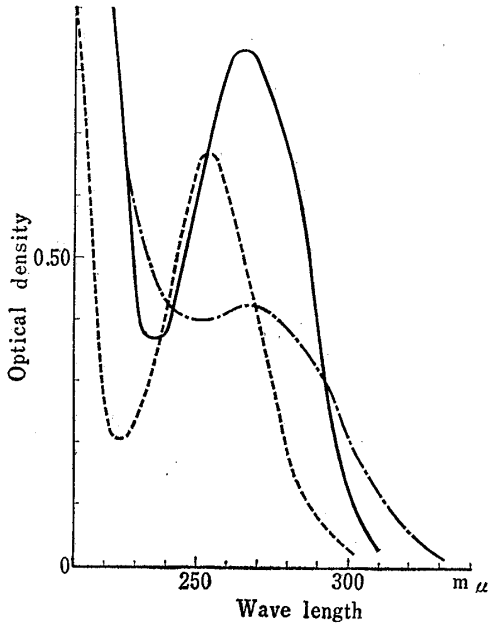

Fig. 1. Ultraviolet Absorption Spectra of Benzohydroxamic Acid and Benzaldoxime

——— Benzohydroxamic acid ($1\times10^{-4}M$) in $0.1M$ borate buffer (pH 9.5)
– – – – Benzohydroxamic acid ($1\times10^{-4}M$) in $0.1M$ phosphate buffer (pH 9.5)
-------- Benzaldoxime ($2\times10^{-5}M$) in $0.1M$ phosphate buffer (pH 9.5)

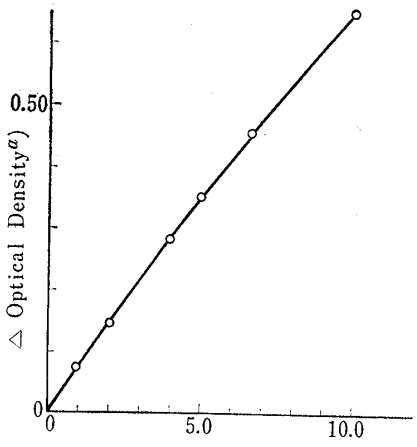

Fig. 2. Calibration Curve of Benzohydroxamic Acid (I)

a) Difference in optical density at 265 mμ between I in $0.1M$ borate buffer (pH 8.3) and in $0.1M$ phosphate buffer (pH 8.3).

*1 The outline of this note was read at the 37th annual meeting of Japanese Biochemical Society at Nagoya in 1964.

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1) J. Hase, K. Kobashi : Unpublished data.
2) C. A. Zittle : Advances in Enzymology, **12**, 493 (1951).
3) A. L. Green : J. Org. Chem., 2566 (1956).

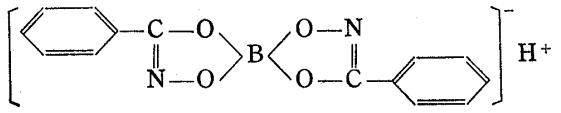
plexe of isonicotinohydroxamic acid and boric acid spectroscopically and titrimetrically, but did not isolate such a complex yet. In this note, we demonstrated the formation of a benzohydroxamic acid-boric acid complex by spectrophotometric measurement and the ratio of I to boric acid to be two by chemical analysis of the isolated complex.

Fig. 1 shows ultraviolet absorption spectra of I in borate and phosphate buffer comparing with that of benzaldoxime in phosphate buffer at the same pH (9.5). The optical density of I at 265 mμ remarkably increases in borate buffer. The peak absorption of benzaldoxime and acetophenon oxime*[3] is 252 and 245 mμ respectively at the same condition, and both peaks were not affected by borate. The resemblance of absorption spectrum and the peak of I in borate buffer with those of benzaldoxime and acetophenon oxime strongly suggests that I is such one form of tautomer as C_6H_5-C(OH)=N-OH in borate solution, which is a resemble structure of benzaldoxime and acetophenon oxime, and then forms probably a cyclic complex with boric acid.

The relationship between the optical density and the concentration of I in excess amount of borate is shown in Fig. 2. I was able to be determined with higer sensitivity by measuring its extinction at 265 mμ than the method of Lipmann and Tuttle.[4]

Boric acid complexes, in general, are known to be highly dissociable in aqueous solution. Benzohydroxamic acid-boric acid complex could not be isolated from the solution in borate buffer. According to the method of the preparation of mannose-borate complex,[5] a mixture of 5.0 g. of I and 1.15 g. of boric acid in 30 ml. of abs. ethanol was heated on a water bath under reflux for 2 hr., and then the solution filtrated stood for a few days in a refrigerator to isolate white needles, decomposed at 258°. The result of analysis of the complex as shown in Table I supports that the molar ratio of I to boric acid in the crystalline complex is 2:1. This result, however, does not necessarily mean the complex isolated from the ethanol solution to be identical with that in aqueous solution.

Table I. Analytical Data of Benzohydroxamic Acid-Boric Acid Complex



Component acid[a]	Exp. No. 1	No. 2	No. 3	Average	Molar ratio (Benzohydroxamic / Boric)
	(μmoles/mg.)				
Benzohydroxamic	7.23	7.18	7.20	7.20	1.98
Boric	3.55	3.72		3.64	

$C_{14}H_{10}O_4N_2B$ *Anal.* Calcd. : C, 59.61; H, 3.93; N, 9.93; ash as B_2O_3, 3.8.
Found : C, 58.36; H, 4.04; N, 8.91; ash 3.1.

a) Benzohydroxamic acid and boric acid were determined by the method of Lipmann[4] and of Miyamoto using curcumin (M. Miyamoto : Bunseki kagaku, **11**, 635; **12**, 115) respectively.

Aliphatic hydroxamic acids are also assumed to form corresponding complexes abovementioned with boric acid, from the observation of the effect on urease inhibition.

*3 Benzaldoxime and acetophenon oxime were synthesized by the method of Hauser, *et al.* (Org. Syntheses, **19**, 15) and of Beckman (Ber., **23**, 1684), and melted at 125° and at 35°(α-form).

4) Lipmann, L. C. Tuttle : J. Biol. Chem., **159**, 21 (1945).
5) J. J. Fox, A. J. H. Gauge : J. Chem. Soc., **99**, 1075 (1911).

However, the marked change in their absorption spectrum by the addition of borate could not be observed and the complex could not be isolated in the similar way.

The authors are grateful to Mr. S. Ogawa for his assistance in this study and to Mr. M. Morikoshi of this Faculty for elemental analysis.

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Kenji Suzuki and Takashi Abiko: Synthesis of 3-L-Lysine-bradykinin and Its O-Acetyl Compound.*1

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The potentiating effect of L-arginyl-L-prolyl-L-lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine (I) corresponding to positions 13 to 21 of B-peptide of ox co-fibirin upon the bradykinin-induced contraction of isolated mouse ileum have been reported in a previous paper.[1] In a comparison between the amino acid sequence of I and bradykinin, L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine (II), as shown in Fig. 1, it is noticed that L-lysine residue of 3-position of I has a basic amino group in the side chain, L-serine residue of 6-position of II has a hydrophylic hydroxyl group, and the other side chains between the N- and the C-terminal arginine residue of I and II have hydrophobic groups. The synthetic 6-L-leucine bradykinin[2] in which the hydrophylic hydroxymethyl group in 6-position of II, was substituted for hydrophobic isobutyl group showed no potentiating effect upon the bradykinin-induced contraction of isolated mouse ileum and practically no bradykinin-like activity.

In the present paper, the synthesis of 3-L-lysine bradykinin is reported to elucidate whether the substituted L-lysine residue in 3-position of bradykinin contributes toward potentiating effect upon bradykinin-induced contraction of isolated guinea pig ileum. The method employed for the synthesis of 3-L-lysine bradykinin is essentially the same as described in the preparation of bradykinin analogs,[3] and ox co-fibrin peptide fragment.[1] N-Benzyloxycarbonylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester[4] was de-benzyloxycarbonylated with hydrogen bromide-acetic acid solution in the presence of anisole and the resulting pentapeptide ester was condensed with N^{α}-*tert*-butyloxycarbonyl-N^{ω}-benzyloxycarbonyl-L-lysine *p*-nitrophenyl ester[1] to yield N^{α}-*tert*-butyloxycarbonyl-N^{ω}-benzyloxycarbonyl-L-lysylglycyl-L-phenylalanyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl

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*1 Nomenclature of bradykinin analogues followed those given in Proc. 2nd. Intl. Pharmcol. Meeting, Vol. 10 Oxytocin, Vasopressin, and their Structual Analogues. Ed. J. Rudinger. xi (1964). Czechoslovak Medical Press, Praha. Abbreviations for amino acids and substituents followed those in given in the tentative rules of IUPAC-IUB commission on biochemical nomenclature, Biochemistry, **5**, 2485 (1966).

*2 Nankozawa, Sendai (鈴木謙次, 安孫子 敬).

1) K. Suzuki: This Bulletin, **14**, 909 (1966).
2) K. Suzuki, M. Asaka, T. Abiko: *Ibid.*, **14**, 211 (1966).
3) K. Suzuki, T. Abiko, M. Asaka: *Ibid.*, **14**, 217 (1966).
4) K. Suzuki, T. Abiko: *Ibid.*, in press. Synthesis of 4-L-valine-6-L-threonine-, 4-L-isoleucine-6-L-threonine-, 4-L-leucine-6-L-threonine-, 4-L-valine-, 4-L-isoleucine-bradykinin and their O-acetyl compounds.