TABLE III. Binding of Radiocalcium by Soluble Fraction from Normal Rat Liver

Treatment	Protein (mg/ml supernatant)	Radiocalcium binding activity[a]	
		S/R net (ml supernatant)	S/R net/mg protein (ml supernatant)
None	1.05	0.097[b]	0.092
Heat-treated	0.28	0.091	0.325

a) Calcium binding determined by Chelex-100 resin assay, and calcium binding activity was expressed as

$$S/R \text{ net} = \frac{^{45}\text{Ca in test supernatant (\%)}}{^{45}\text{Ca in test resin (\%)}} - \frac{^{45}\text{Ca in blank supernatant (\%)}}{^{45}\text{Ca in blank resin (\%)}}$$

b) A duplicate experiment gave the same relative data.

The present results clearly demonstrate that the calcium-binding activity exists in the soluble fraction of a rat liver. Gel filtration studies with Sephadex G-75 further suggested that the activity is associated with a protein.[11] Presumably, calcium-binding protein in the soluble fraction of a rat liver is involved in the transport of calcium in the liver cells.

11) M. Yamaguchi and T. Yamamoto, "submitted."

[Chem. Pharm. Bull. 23(10)2421—2424(1975)]

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Benzodiazepines. XI.[1] Further Examination of the Chromic Acid Oxidation of 2-Aminomethylindoles to 2,3-Dihydro-2*H*-1,4-benzodiazepin-2-ones

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In the chromic acid oxidation of 2-aminomethyl-5-chloro-1-methyl-3-phenylindole (**1**) to 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one (**2**), **1** reacts rapidly with chromic acid to form an oxidation intermediate A, which on acetylation with acetic anhydride gives 2-acetamido-2′-benzoyl-4′-chloro-N-methylacetanilide (**3**). The rate for formation of **2** from A has been determined by following the change of the quantities of **2** and **3** obtained by periodical sampling followed by quenching with acetic anhydride. The rate is first order with respect to the concentration of A.

In the preceding papers of this series the oxidative ring enlargement of 2-aminomethylindoles to 1,3-dihydro-2*H*-1,4-benzodiazepin-2-ones have been reported.[3] The present study was undertaken in order to consider more closely the mode of formation of 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one (**2**) by the chromic acid oxidation of 2-aminomethyl-5-chloro-1-methyl-3-phenylindole (**1**).

1) Part X: K. Ishizumi, K. Mori, S. Inaba, and H. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **23**, 2169 (1975).
2) Location: *2-1, Takatsukasa-4-chome, Takarazuka-shi, Hyogo.*
3) *a*) H. Yamamoto, S. Inaba, T. Hirohashi, and K. Ishizumi, *Chem. Ber.*, **101**, 4245 (1968); *b*) S. Inaba, T. Hirohashi, and H. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **17**, 1263 (1969); *c*) S. Inaba, K. Ishizumi, and H. Yamamoto, *ibid.*, **19**, 263 (1971); *d*) S. Inaba, K. Ishizumi, K. Mori, and H. Yamamoto, *ibid.*, **19**, 722 (1971).

The oxidation of **1** with chromic acid in acetic acid, followed by an alkaline work-up, was examined at 30° with a reaction time of 2 hr using various ratios of the oxidant to **1**. As shown in Table I, the use of a 3-fold excess of the oxidant was sufficient to ensure complete reaction.

Table I. Oxidation of 2-Aminomethylindole Hydrochloride **1**[a] with Various Molar Ratios of Chromic Acid[b]

Molar Ratio (CrO_3/1)	1	2	2.5	3	3.5	4
Yield, %	32	70	79	91	89	90

a) 3.26 mmoles *b*) The reaction time was 2 hr at 30°.

Preliminary experiments revealed that **1** reacted rapidly with chromic acid to form the intermediate A[4] which on treatment with acetic anhydride gave 2-acetamido-2′-benzoyl-4′-chloro-N-methylacetanilide (**3**),[6] and the subsequent product-forming step was rate determining (Chart 1). The rate at which A forms **2** was examined. For following the reactions, the method of quenching with acetic anhydride[6] was employed assuming that the trapping of A as **3** is quantitative. To make sure that the formed product **2** is inert under the conditions used for quenching of the reaction mixture, **2** was subjected to identical acetylating conditions, and was recovered unchanged.[7]

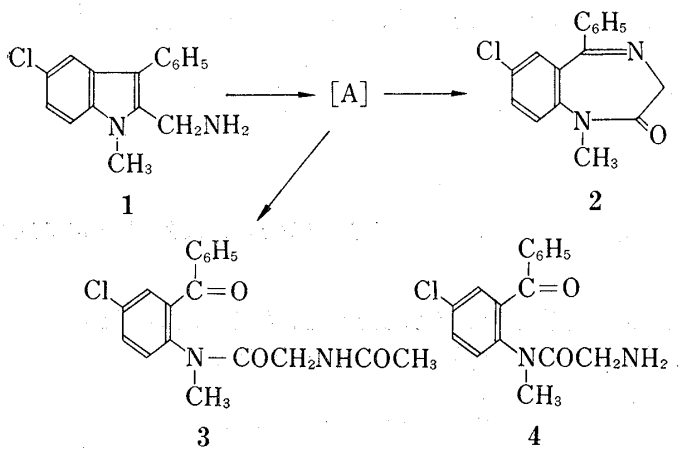

Chart 1

The oxidation of the hydrochloride of **1** was carried out in acetic acid at 31.0° using a 3.5-fold excess of chromic acid. Samples were removed at intervals and the reaction was quenched with acetic anhydride. The products **2** and **3** were separated by preparative layer chromatography and analyzed by ultraviolet (UV) spectroscopy. The change in yields of **2** and **3** with time is given in Fig. 1. The sum of **2** and **3** obtained during the first three hours of the reaction was nearly constant except for the first point. This average value was used as the initial concentration of A. First-order rate plots were obtained assuming $-d[A]/dt=k[A]$ as shown in Fig. 2. In plotting log Ao/At against time Ao stands for the initial concentration of A and At is the concentration of A remaining in the reaction mixture at time t. The rate constant obtained from the slope of the kinetic curves is indicated in Table II.

The oxidation of the hydrochloride of **1** proceeded without formation of a precipitate although the oxidation of the hydrochloride of 2-aminomethyl-1-methyl-5-nitro-3-phenylindole precipitated directly a chromic acid salt of the corresponding benzodiazepin-2-one.[3d] However, when the reaction mixture was diluted with water, the dihydrochromate of **2** gradually

4) Although it is tempting to speculate the oxidation intermediate A to be 2-amino-2′-benzoyl-4′-chloro-N-methyl acetanilide (**4**),[5] several other structures are possible for A. We are continuing our studies on the nature of A in detail.

5) *a*) R.J. Sundberg, "The Chemistry of Indoles," Academic Press, New York, N.Y., 1970; *b*) G.N. Walker, A.R. Engle, and R.J. Kempton, *J. Org. Chem.*, **37**, 3755 (1972).

6) Private communication from Hoffmann-La Roche Ltd., Basle, Switzerland.

7) The formation of 3-acetamido-6-chloro-1-methyl-4-phenyl-2(1*H*)-quinolone by refluxing of **2** with acetic anhydride in the presence of sodium acetate has been reported: R.I. Fryer and L.H. Sternbach, *J. Org. Chem.*, **30**, 524 (1965).

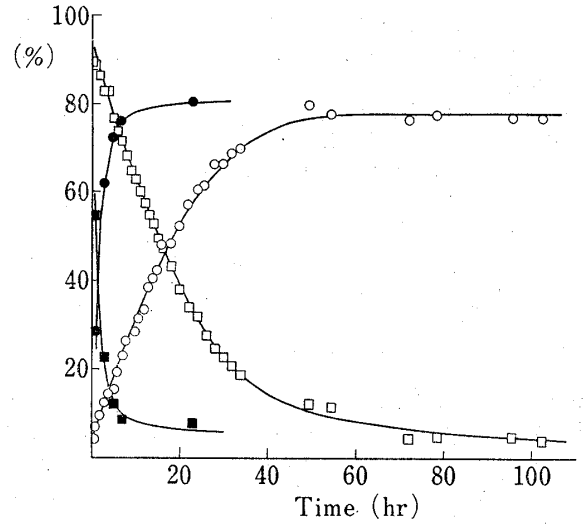

Fig. 1. Formation of **2** from the Intermediate A formed by Reaction of the Hydrochloride of **1** with a 3.5-fold Excess of Chromic Acid at 31.0° in the Presence (●,■) and Absence (○,□) of Chromic Acetate

○,●=2 □,■=3

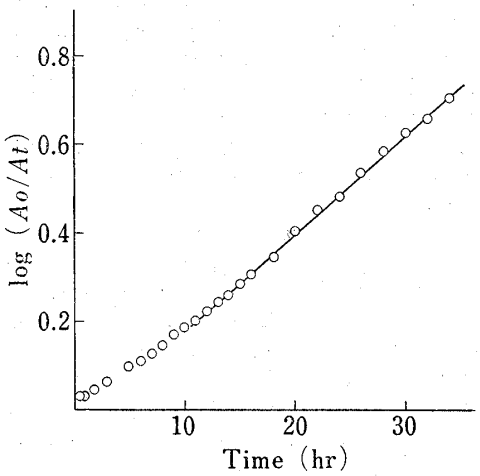

Fig. 2. First-order Plot for the Formation of **2** from the Intermediate A formed by Reaction of the Hydrochloride of **1** with a 3.5-fold Excess of Chromic Acid

precipitated in 61% yield. This compound was identified by direct comparison with a sample prepared from the hydrochloride of **2** by treatment with chromic acid in acetic acid.

In order to examine the effect of chromic acid concentration on the rate for formation of **2**, the oxidation of **1** was repeated using a lower concentration (a 3-fold excess) of chromic acid. As shown in Table II the decrease of chromic acid concentration accelerates rather than retards the formation of **2**. This may be explained on the assumption that the rate

Table II. Rate of Formation of Benzodiazepine **2** from Chromic Acid Oxidation of 2-Aminomethylindole Hydrochloride **1** at 31.0° in 92% Acetic Acid

Molar ratio CrO_3/**1**	Molar ratio $Cr(OAc)_3$/**1**	k. sec^{-1}
3.0	—	2.6×10^{-5}
3.5	—	1.4×10^{-5}
3.5	10.5	1.2×10^{-4}

of formation of **2** increases with decreasing acidity of the reaction mixture since chromic acid is known to cause a marked increase in the acidity of the acetic acid solution.[8] This reasoning is supported by the fact that the formation of **2** is accelerated by a factor as large as 8.6 by the addition of chromic acetate which decreases the acidity of an acetic acid solution of chromic acid even below that of pure acetic acid[8] (Figure 1, Table II).

Experimental

Melting points were determined in open capillary tubes and are uncorrected. The UV spectra were measured in methanol on a Shimadzu D-40 spectrophotometer and infrared (IR) spectra on a Hitachi Model EPI-G3 spectrometer. Preparative layer chromatography was done on 0.5 mm layers of Merck Silica gel HF_{254} using ethyl acetate–chloroform (5 : 3) as eluent.

Purification of 2-Aminomethyl-5-chloro-1-methyl-3-phenylindole (1)——Crude hydrochloride of **1** was prepared according to the procedure described earlier.[3a] Samples were purified as the free base by recrystal-

8) Y. Ogata, A. Fukui, and S. Yuguchi, *J. Amer. Chem. Soc.*, **74**, 2707 (1952).

lizations from isopropyl ether. The pure base (40 g), mp 74—77°, was dissolved in 160 ml of ethanol, and 40 ml of 18% ethanolic hydrogen chloride was added. The precipitate was collected by filtration to give 40 g of the pure hydrochloride of **1**, mp 250—253° (decomp.).

Chromic Acid Oxidation of Hydrochloride of 1 Followed by an Alkaline Work-up——The following procedure is typical of the experiments carried out to determine the yields of **2** with varying quantities of chromic anhydride. To a solution of 0.98 g of chromic anhydride in a cold mixture of 0.8 ml of water and 10 ml of acetic acid was added, in portions, 1.0 g of the pulverized hydrochloride of **1** with vigorous stirring over a period of 10 min. After stirring at 30 0° for 2 hr, 10 ml of water followed by 10 ml of ether was added, and the mixture was cooled and made basic with 40% sodium hydroxide solution. The ether layer was separated and the aqueous layer extracted with ether. The combined organic layers were dried over sodium sulfate and evaporated under reduced pressure to give 0.86 g of the crude **2**. The product was purified by preparative layer chromatography and its quantity was determined by UV spectroscopy. The results of this and similar experiments are summarized in Table I.

Rate of Formation of 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one (2) from Intermediate A Formed by Reaction of Hydrochloride of 1 with a 3.5-fold Excess of Chromic Acid——(a): To a solution of 28.5 g of chromic anhydride in 22.8 ml of water and 250 ml of acetic acid that cooled to 9° in an ice bath was added, in portions and with vigorous stirring, 25 g of the pulverized hydrochloride of **1** over a period of 10 min. During the addition the temperature rised gradually to 28°. The reaction flask was immersed in a thermostated bath at 31.0° and allowed to come to thermal equilibrium for 3 min. The time was taken as time zero, when thermal equilibrium was reached. Aliquots (6.0 g) were periodically withdrawn, diluted with 30 ml of methylene chloride, and cooled in an ice-salt mixture. The resulting mixtures were acetylated by adding 5.9 ml of acetic anhydride followed by 23.5 ml of 40% sodium hydroxide solution. During the addition of sodium hydroxide solution the temperature was kept below 5°. To the acetylated mixture was added 50 ml of water in order to dissolve precipitated sodium acetate. The methylene chloride layer was separated, the aqueous layer was extracted with methylene chloride, and the combined organic layers were washed with water, dried over sodium sulfate and concentrated under reduced pressure. The products **2** and **3** were separated by preparative layer chromatography and their quantities analyzed by UV spectroscopy.

The same procedure was used for the reaction with a 3.0-fold excess of chromic acid. First-order rates were calculated graphically (Table II).

In the above reaction with a 3.5-fold excess of chromic acid, after 105 hr an 6.0 g-aliquot was withdrawn and diluted with 10 ml of water. The solution was decanted from a small amount of qummy material and allowed to stand at room temperature for 4 days. The precipitate was collected by filtration to give 300 mg of the dihydrochromate of **2**, mp 159—163° (decomp.). From the mother liquor an additional 52 mg of product, mp 164—165 (decomp.), was obtained to give a combined yield of 352 mg (60.5%). Recrystallization from aqueous acetic acid afforded orange prisms, mp 164—167° (decomp.). The IR spectrum was identical with that of an authentic sample prepared below from the hydrochloride of **2** and chromic acid.

(b) With Added Chromic Acetate: To a cooled solution of 3.42 g of chromic anhydride in 2.7 ml of water and 30 ml of acetic acid was added, in portions, 3.0 g of the pulverized hydrochloride of **1** with stirring over a period of 10 min. During the addition the temperature rised to 16°. After stirring for another 10 min at room temperature, 25.4 g of hydrated chromic acetate was added in one portion at 22°, and the mixture was allowed to equilibrate in a thermostated bath at 31.0° Sampling, work-up, and analytical procedures were taken in the same manner as given in a.

Analysis——The quantities of compounds **2** and **3** separated by preparative layer chromatography were determined by comparison of the UV absorption intensities at 314 mμ (**2**) and 252 mμ (**3**) with those of standard solutions.

Authentic Dihydrochromate of 2——To a solution of 0.42 g of chromic anhydride in 0.4 ml of water and 5 ml of acetic acid was added 0.50 g of the hydrochloride of **2** at 15°. After stirring at room temperature for 3 hr, the mixture was diluted with 15 ml of water. The solution was decanted from gummy material and allowed to stand at room temperature for 2 weeks. The precipitate formed was collected by filtration to give 336 mg (53.6%) of the dihydrochromate of **2**, mp 164—167° (decomp.). Recrystallization from aqueous acetic acid afforded orange prisms: mp 164—167° (decomp.). IR ν_{max}^{Nujol} cm^{-1}: 1720, 1704 (CO), 1640 (C=N), 1600, 945 and 920 (CrO_4^{2-}). *Anal.* Calcd. for $C_{16}H_{15}O_5N_2ClCr$: C, 47.72; H, 3.76; N, 6.95; Cl, 8.80. Found: C, 47.78; H, 3.55; N, 6.89; Cl, 8.26.

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