Frakt. 3 : $C_{26}H_{29}O_3N_2Cl \cdot \frac{1}{2}H_2O$—Ber. C, 67.60; H, 6.50; N, 6.06. Gef. : C, 67.70; H, 6.78; N 6.06. Eine kleine Probe gab beim Verseifen mit 15-proz. äthanolischer Salzsäure Nädelchen vom Schmp. 103° (auftauend bei 90°). $C_{19}H_{26}O_3N_2 \cdot \frac{1}{2}H_2O$—Ber. : C, 68.10; H, 7.90; N, 8.30. Gef. : C, 68.07; H, 8.12; N, 7.29.

Frakt. 4 : Durch eine Mischprobe mit (XI) identifiziert.

2′-Chlor-O,*ac*-N-dibenzoyldihydronichin (XII)—6 g (IX) wurden in 90 ccm $CHCl_3$ mit 6 g $POCl_3$ 4 Std. unter Rückfluss erhitzt. Die Reaktionsmischung wurde zuerst mit Eis, dann mit 10-proz. Na_2CO_3-Lösung zersetzt und die $CHCl_3$-Schicht nach dem Trocknen mit Na_2SO_4 eingedampft. Der Rückstand erstarrte kristallinisch; er wurde aus Aceton umkristallisiert wurde. Nadeln vom Schmp. 168°. Die Ausbeute : 5.6 g. $C_{33}H_{33}O_4N_2Cl \cdot \frac{1}{4}H_2O$—Ber. : C, 70.59; H, 5.90; N, 4.99. Gef. : C, 70.64; H, 6.22; N, 4.91.

2′-Hydroxydihydronichin (XIV)—i) 0.8 g (XIII) wurden mit 50 ccm 15-proz. äthanolischer HCl 5 Std. unter Rückfluss im Sieden gehalten. Nach dem Erkalten wurde die Reaktionsmischung im Vakuum eingedampft und der Rückstand 3 mal mit Äther durch Digerieren gewaschen. Der unlösliche Teil wurde in 10-proz. NaOH gelöst, filtriert und das Filtrat mit CO_2 gesättigt. Die hierbei ausgeschiedene Masse ergab beim Umkristallisieren aus Aceton-Wasser 0.1 g Nadeln vom Schmp. 204°. $C_{19}H_{26}O_3N_2 \cdot H_2O$—Ber. : C, 65.49; H, 8.10; N, 8.04. Gef. : C, 65.10; H, 7.27; N, 7.99. U. V. λ_{max}^{EtOH} mμ(log ε): 351 (3.79), 235(4.57).

Sulfat : Nadeln aus Aceton-Wasser, Zers. Pkt. 226°. $C_{19}H_{26}O_3N_2 \cdot \frac{1}{2}H_2O$—Ber. : C, 57.70; H, 7.30; N, 7.06. Gef. : C, 58.11; H, 7.79; N, 6.88.

ii) 4 g (XII) wurden in 20 ccm AcOH gelöst, 20 ccm konz. HCl zugesetzt und im Ölbad bei 140～150° 5 Std. unter Rückfluss erhitzt. Nach dem Erkalten wurde die Reaktionslösung auf überschüssiges mit Eis zugesetztes konz. Ammoniak unter Umrühren gegossen. Die ausgeschiedene Base wurde abgesaugt, mit Wasser gewaschen, getrocknet und aus Aceton umkristallisiert. Nadeln vom Schmp. 207°. Die Ausbeute : 0.8 g.

Die Mutterlauge ergab eine syrupöse Masse, die mit 20 ccm 10-proz. äthanolischer KOH Lösung unter Rückfluss 2 Std. erhitzt wurde. Die Reaktions-Lösung wurde eingedampft, der Rückstand in 10-proz. KOH gelöst, filtriert und das Filtrat mit CO_2 gesättigt. Der Niederschlag ergab bei analoger Aufarbeitung 0.8 g Nadeln vom Schmp. 207°. Die gesamte Ausbeute war 1.6 g. Eine Mischprobe mit dem Präparat vom Schmp. 204° schmolz bei 207°.

Zusammenfassung

Dihydronichin wurde nach der Benzoylierung mit Wasserstoffperoxyd in Eisessig-Lösung oxydiert. Das entstandene Mono- und Dibenzoyldihydronichin-N-oxyd wurde durch Behandlung mit phosphoroxychlorid in Chloroform-Lösung in die entsprechenden 2′-Chlordihydronichin-Derivate übergeführt. Die beiden letzteren gaben beim Verseifen das 2′-Hydroxydihydronichin.

(Eingegangen am 17. Januar, 1958)

UDC 615.786-011

49. Hisashi Nogami, Masayoshi Horioka, Shoji Awazu, and Hideo Yamada†: Studies on Decomposition and Stabilization of Drugs in Solution. II.[1] Chemical Kinetic Studies on Aqueous Solution of Methantheline Bromide.

(*Faculty of Pharmaceutical Sciences, University of Tokyo***)

Methantheline Bromide (M. B.), 2-diethylaminoethyl xanthene-9-carboxylate methobromide, is a parasympathetic blocking agent and distributed in the marketed dosage form of tablets or as a sterilized powder for injection. It is indicated in the Dispensatory of U. S. A. that M. B. is unstable in aqueous solution and the use of sterilized

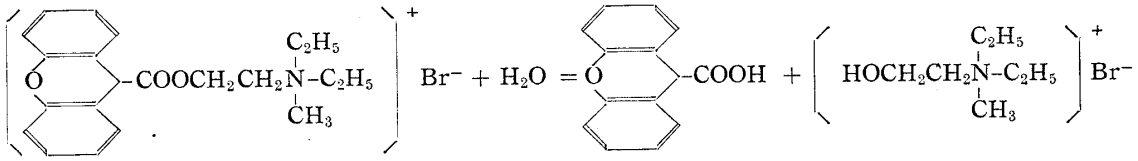
** Hongo, Tokyo (野上 寿, 堀岡正義, 栗津荘司, 山田秀雄).
† Present adress : Shionogi & Co., Osaka.
1) Part I. J. Hasegawa, K. Ikeda, T. Matsuzawa : This Bulletin, **6**, 36(1958).

powder for injection may also show the unstability of the drug. However no work has been published on the unstability of this drug.

It is stated in the U. S. Dispensatory that the formation of xanthene-9-carboxylic acid crystals is used for its identification, decomposing with alkali. Considering these statements and its molecular formula, the decomposition may be due to the hydrolysis of its ester linkage and this assumption was proved spectrophotometrically.

It is the purpose of this investigation to discuss the decomposition of M. B. in aqueous solution from the standpoint of chemical kinetics. This procedure was very effective in the study of hydrolytic decomposition of Aspirin,[2] procaine,[3] phenobarbital,[1] etc.

It may be considered that M. B. is present in an ionized form in aqueous solution through practically all ranges of pH value, because it is a quaternary ammonium bromide. The decomposition may be represented as follows :



Most of the methods reported in the literature for the determination of this drug, however, cannot be used for the following reasons. Sangra[4] proposed a gravimetric method with reineckate which procedure was too complicated and not suitable for frequent determination. Baltazar[5] determined bromine ion by titration in his method, but this also cannot be used, because decomposed substance liberated the same ion. The assay methods described in Tests and Standards for N. N. R., in which bromine ion or nitrogen content is determined, are also unsuitable for the same reason. The accuracy of ultraviolet absorption method is not sufficient, because the characteristic curves of M. B. and its decomposed products are nearly the same as will be described later.

Lehman[6] reported that the minimum amount of basic substance can be determined colorimetrically from body fluid using suitable conditions of extraction and formation of complex compound with acidic dye. Horioka[7] recently examined the condition of the assay method for many basic medicaments using complex formation with dye, and optimum pH value of formation and extraction of complex compound with an organic solvent. He also proved that by selecting suitable conditions, an amine can be determined selectively from a mixture of several basic substances.

This procedure was applied for the present investigation and the selective determination of M. B. from the decomposed substances was effected. Details of the condition examined will be described in the following report.[8]

Theoretical

Judging from the results obtained and the ultraviolet absorption of M. B. and decomposed solution, the velocity of decomposition is represented as follows :

$$-\frac{d[\text{M. B.}]}{dt}=\{k_H[H^+]+k_{OH}[OH^-]+k_{H_2O}[H_2O]\}[\text{M. B.}] \qquad (1)$$

where $[H^+]$, $[OH^-]$, $[H_2O]$, and [M. B.] are the concentration of individuals and k_H, k_{OH},

2) L. Edwards : Trans. Faraday Soc., **46**, 723(1950).
3) T. Higuchi, A. Havinga, L. Busse : J. Am. Pharm. Assoc., **39**, 405(1950).
4) C. Sangra, R. Parreno : Rev. asoc. bioquim. arg., **19**, 87(1954) (C. A., **48**, 13545a(1954)).
5) J. Baltazar, A. Nogueira : Rev. port. farm., **2**, 161(1952) (C. A., **47**, 5310d(1953)).
6) R. Lehman, T. Aikten : J. Clin. Med., **28**, 787(1942).
7) M. Horioka : Yakugaku Zasshi, **77**, 200, 206(1957).
8) Part III. H. Nogami, N. Nakajima : This Bulletin, **6**, 283(1958).

and k_{H_2O} the catalytic constant of H^+, OH^-, and H_2O, respectively. The apparent velocity constant of the reaction is:

$$k = k_H[H^+] + k_{OH}[OH^-] + k_{H_2O}[H_2O]$$
$$= k_H[H^+] + \frac{k_{OH}K_W}{[H^+]} + k_{H_2O}[H_2O] \quad (2)$$

where K_W is the ionization constant of water. Eq. (2) may be changed at the lower and higher pH regions as follows:

$$[H^+] \gg [OH^-] \quad \log k = \log k_H - pH \quad (3)$$
$$[H^+] \ll [OH^-] \quad \log k = \log k_{OH} + pH + \log K_W \quad (4)$$

From the Eqs. (3) and (4), linear relationship between $\log k$ and pH is expected in these regions.

Between these regions the velocity will pass through a minimum at a hydrogen ion concentration which is found by differentiating k with respect to $[H^+]$ in Eq. (2) and equating to zero, thus:

$$[H^+]_m^2 = K_W \frac{k_{OH}}{k_H} \quad \text{or} \quad (pH)_m = -\frac{1}{2}\{\log K_W + \log k_{OH} - \log k_H\} \quad (5)$$

where $[H^+]_m$ and $(pH)_m$ represent the hydrogen ion concentration and pH at minimum velocity, respectively.[9]

Experimental

Ultraviolet Absorption Spectra—Ultraviolet absorption of the following solutions are shown in Fig. 1. Curve (a) is one of M. B. solution immediately after preparation and (b) is one of M. B. after storage for 5 days at 40° in the buffer solution of pH 10 in which the decomposition might be complete. Curve (c) is one of the equimolar solution of synthesized diethylaminoethanol methobromide and the acidic substance, m.p. 218~220°, which is extracted by $CHCl_3$ from the M. B. solution completely decomposed by alkali. *Anal.* Calcd. for $C_{14}H_{10}O_3$ (Xanthene-9-carboxylic acid): C, 74.3; H, 4.46. Found: C, 74.4; H, 4.16.

Colorimetry—1. Reagents: All reagents used in this study were extra pure grades and M. B. was offered by the Dainippon Seiyaku Co.

0.1% Bromocresol purple (BCP)—0.1 g. BCP was well ground in a glass mortar with 9.25 cc. of 0.02*N* NaOH and diluted to 100 cc. with distilled water.

Buffer solution of pH 6.5—Twice concentrated Clark-Lubs' buffer solution was used.

Ethylene dichloride (EDC)—Washed with dilute alkali and aqueous acid solution, and distilled after drying.

0.1*N* NaOH—4 g. of NaOH was dissolved in 1000 cc. of distilled water.

2. Procedure: 20 cc. of EDC, 10 cc. of the buffer solution of pH 6.5, and 1.0 cc. of BCP solution are added to 1.0 cc. of the test solution in a glass-stoppered test tube, shaken vigorously for 5 mins., and centrifuged for 5 mins. The aqueous layer is taken off by suction, 10 cc. of EDC layer is transferred into another test tube, and 10 cc. of 0.1*N* NaOH is added. The tube is shaken, centrifuged as described above, and the colored aqueous layer submitted to colorimetry with Beckman spectrophotometer Model DU at 591 mμ. The linear regression was calculated from the determinations of 12 points. The calculation formula: $Y = 7.029X + 21.4$, where Y is the optical density $\times 10^3$ and X the amount (in γ) of M. B.

3. Preparation of samples: M. B. was dissolved to make ca. 0.01% solution in the specified buffer solutions of various pH values studied and poured into 5-cc. ampules. These ampules were stored in a thermostatically controlled water bath adjusted to 62.5°±0.2°, 52.0°±0.4°, and 37.5°±0.2°, and ice-water bath. The ampules were taken out periodically at given intervals during the experiment, cooled immediately with ice and water, and 1 cc. of the content was used for the colorimetry as described above. The buffer solution used as the vehicle was prepared according to the prescription of Clark-Lubs, and Sörensen in the region of above pH 10. The values of pH were determined with Beckmann pH-meter model G.

Results and Discussion

It may be considered that the perfect identity of the curves (b) and (c) in Fig. 1 supports the assumption that the decomposition of M. B. is hydrolysis of an ester.

9) A. Kondritzer, P. Zvirblis: J. Am. Pharm. Assoc., **46**, 531(1957).

In all cases observed, the rate of decomposition of M. B. followed a first-order course. The typical results obtained are shown in Fig. 2.

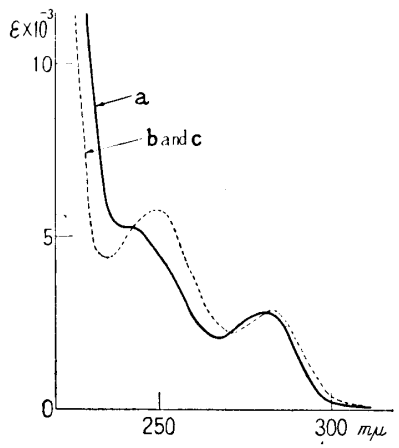


Fig. 1.
Ultraviolet Absorption Spectra of M. B. and Its Decomposition Product (Unicam SP-500)

a : M. B. solution immediately after preparation
b : M. B. solution after storage for 5 days at 40° in the buffer solution of pH 10
c : Equimolar solution of synthesized diethylaminoethanol methobromide and $CHCl_3$-extract of decomposed M. B. solution

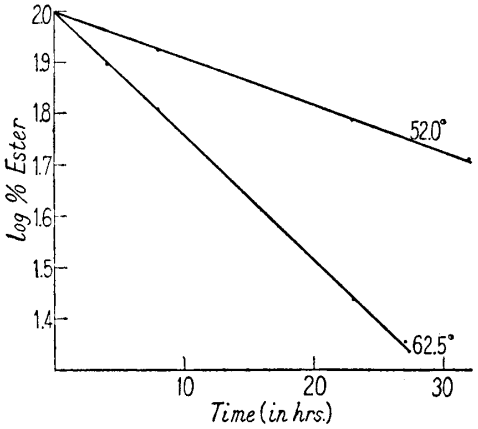


Fig. 2. First-order Disappearence of M. B. at pH 6.12

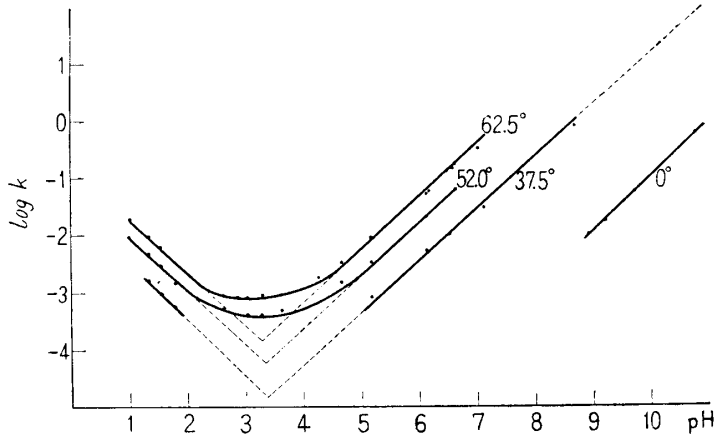


Fig. 3. Relationship between log k and pH at each Temperature

The apparent velocity constant k is calculated as follows :

$$k=\frac{2.303}{t}\log\frac{a}{a-x}$$

where t is time in hrs., a the initial concentration of M. B., and x the amount decomposed.

The log k is plotted against pH in Fig. 3, in which the experiments between pH 2 and 5 at 37.5° were not carried out, because M. B. decomposes very slowly at that pH. As mentioned in the theoretical discussion, the relationship between pH and log k is linear in the relatively low and high pH regions, and the minimum is observed between them. The velocity constants determined from the experiment at which 0.5 mole/L. of NaCl was added in the buffer solution of pH about 6.5, showed the salt effect to be negligible.

The concave is not so sharp near the minimum point in Fig. 3. The result may show the presence of water catalysis, for it resembles the type of hydrogen ion, hydroxyl ion, and water catalyses discussed by Edwards in his report on Aspirin.[2] The straight lines of both lower and higher pH regions are extended and a cross point is obtained. The difference between the minimum velocity constant experimentally obtained and the double value of the cross point represents the velocity of water catalysis. By this procedure, $k_{H_2O}[H_2O]$ is obtained and tabulated in Table I. By dividing $k_{H_2O}[H_2O]$ with 55.5, k_{H_2O} is obtained and also shown in Table I.

Table I. The Velocity Catalyzed with Water and Its Catalytic Constant

Temp. (°C)	k_{H_2O} (H_2O) (hr.$^{-1}$)	k_{H_2O} (hr.$^{-1}$)
62.5	5.06×10^{-4}	9.12×10^{-6}
52.0	2.64×10^{-4}	4.76×10^{-6}

The linear relationships were recognized between log k and the reciprocal of absolute temperatures in both lower and higher pH regions. The typical results are shown in Fig. 4.

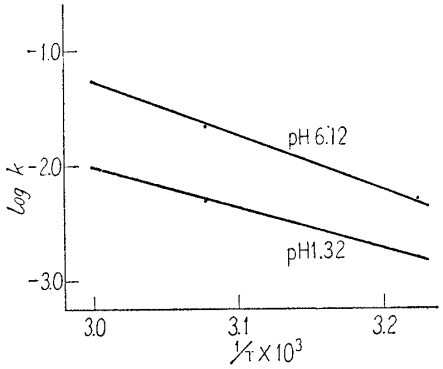


Fig. 4.
Temperature Dependence of Hydrolytic Velocity of M. B.

The frequency factor and activation energy of k_H were obtained directly from Eq. (3) and the Arrhenius equation. In a higher pH region, the apparent velocity constant k is expressed as follows :

$$\log k = 7.35 - \frac{4.88\times10^3}{T} + \mathrm{pH} \qquad (6)$$

log k is related to log k_{OH} and log K_W as shown in Eq. (4). The value of log K_W is expressed by the van't Hoff equation as follows :

$$\frac{d\ln K_W}{dT} = -\frac{\Delta H}{RT^2}$$

Therefore, the value of log K_W as the function of temperature is necessary for the calculation of k_{OH}. Harned[10] reported that the value of pK_W was 13.017, 13.262, and 13.680 at 60°, 50°, and 35°, respectively. Using these values, Eq. (7) is obtained as the usual function of $1/T$.

$$\log K_W = -4.90 - \frac{2.705\times10^3}{T} \qquad (7)$$

By Comparing Eq. (7) with Eqs. (4) and (6) the following equation is obtained.

$$\log k_{OH} = 12.25 - \frac{2.175\times10^3}{T} \qquad (8)$$

For the lower pH region, log k_H is as follows :

$$\log k_H = 9.30 - \frac{3.35\times10^3}{T} \qquad (9)$$

Substituting Eq. (7), (8), and (9) in Eq. (5), the pH value of minimum velocity constant is expressed as follows :

$$(\mathrm{pH})_m = 0.975 + \frac{0.765\times10^3}{T} \qquad (10)$$

The values of $(\mathrm{pH})_m$ calculated with Eq. (10) and cross points in Fig. 3 are shown in Table II.

The velocity constant k in high pH region can be estimated from Eq. (6) which relates to log K_W and log k_{OH}. It may be noted that when dissolving the van't Hoff and

10) H. Harned, R. Robinson : Trans. Faraday Soc., **36**, 973(1940).

Table II. Calculated and Found pH at Minimum Velocity

Temp. (°C)	$(pH)_m$	
	Calcd.	Found
62.5	3.26	3.28
52.0	3.33	3.32
37.5	3.44	3.38

Arrhenius equations, ΔH and activation energy are treated as the constants which do not depend on temprature, but it has been shown by Wörman[11] that ΔH changes with temperature. Then, it may be necessary to examine whether Eq. (6) obtained from higher temperature experiments can be used without any correction to estimate the velocity at a low temperature. For this purpose, the velocity constant at 0° was obtained above pH 8.90 and compared with the values on the extended line at 37.5° in Fig. 3. First, supposing that ΔH does not change with temperature, the difference between $\log k_{37.5}$ and $\log k_0$ was calculated with Eq. (6) without any correction, where $k_{37.5}$ and k_0 are the calculated velocity constants at 37.5° and 0°, respectively. The difference was 2.16. Second, considering that ΔH changes with temperature, the difference was calculated. The average ΔH between 0° and 37.5° is necessary for the calculation. The ΔH obtained was 13.9 kcal. using pK_W by Harned. Substituting 13.9 kcal. in Eq. (7) instead of 12.4 kcal., Eq. (6) is changed as follows :

$$\log k = 8.43 - \frac{5.21 \times 10^3}{T} + \mathrm{pH}$$

The difference was calculated and was 2.30. These values are compared in Table III.

Table III. Difference between $\log k_{37.5}$ and $\log k_0$

pH	Found	$\Delta H = 12.4$ (not corrected)	$\Delta H = 13.9$ (corrected)
8.90	2.24	2.16	2.30
9.20	2.25		
9.70	2.22		
10.79	2.12		

It was expected that the correction was reasonable and more accurate velocity constants would be obtained by the correction, but the results showed that the correction of ΔH was not necessary, at least in this experiment.

The half lives ($t_{0.5}$) at $(pH)_m$ can be calculated by means of the following equation:

$$t_{0.5} = \frac{0.693}{2k_H[H^+] + k_{H_2O}[H_2O]}$$

Giving an instance at 30°, $(pH)_m$ is 3.50 caluculated from Eq. (10) and $t_{0.5}$ may be 545 days, where $k_{H_2O}[H_2O]$ was estimated from the two values at 62.5° and 52.0°, and $t_{0.5}$ at pH 7.0 may be 39.4 hrs. from Eq. (6).

The authors wish to express their thanks to Dainippon Seiyaku Co. for the offer of Methantheline Bromide.

Summary

1. It was evidenced by the ultraviolet absorption spectral analyses that the decomposition of Methantheline Bromide (M. B.) in aqueous solution was the hydrolysis of ester linkage.

2. The determination of M. B. was carried out separating from its decomposed substances by the colorimetric procedure using ethylene dichloride as a solvent, bromocresol purple as a complexing dye, in a buffer solution of pH 6.5.

3. The decomposition was recognized as a first-order reaction at any given pH value and the reaction was catalyzed by hydrogen ion, hydroxyl ion, and water. The constants were determined as follows :

$$k_H = 2.00 \times 10^9 e^{-\frac{15,300}{RT}} \qquad k_{OH} = 1.76 \times 10^{12} e^{-\frac{9,950}{RT}}$$

(Received January 20, 1958)

11) A. Wörman : Ann. Physik., **18**, 775(1905).