U.D.C. 615.781.63-0.14.172 : 541.124

14. **Ken Ikeda** : Equilibrium of Procaine-N-glucoside Formation in Parenteral Solutions containing Procaine and Glucose.

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It is known that primary aromatic amine combines with glucose and forms aryl-N-glucoside in aqueous solution.[1] Sannié and Vincent[2] pointed out that anesthetic activity of procaine injection containing glucose decreases during storage and that this phenomenon is caused by the formation of procaine-N-glucoside (PNG). Cannell[3] also examined the change of optical rotation of various combinations of anesthetic amines and sugars in aqueous solutions and concluded that the change occurs only in solutions of primary amine and aldose. He synthesized PNG and studied its chemical properties. Kaito[4] evidenced by paper chromatography that PNG is formed in a preparation for chilblains containing 1% of procaine hydrochloride and 5% of glucose.

The injection containing 0.5% of procaine hydrochloride and 5% of glucose (PGI) is used for anesthsia in a cardiac operation in the Tokyo University Hospital, and notable decrease of anesthetic activity of this injection during storage was observed. However, there has been no quantitative studies on it in the past. The author investigated the change in this preparation from the standpoint of chemical equilibrium.

The chemical change of formation of PNG can be represented as follows :

$$(C_2H_5)_2NCH_2CH_2OCO\text{-}\phi\text{-}NH_2 + OHC(CHOH)_4CH_2OH = (C_2H_5)_2NCH_2CH_2OCO\text{-}\phi\text{-}NHCH(CHOH)_3CHCH_2OH + H_2O \quad (1)$$

From Kaito's study on its paper chromatography[4] it may be said that there is no other chemical change of procaine to be investigated besides the above reaction. To study the above reaction quantitatively, it is necessary to estimate accurately procaine remainning uncombined in the solution without interferances by other components or by hydrolysis of PNG. Procaine may be estimated by the method described in Japanese Pharmacopoeia, in which procaine is treated with strong alkali and acid.[4] However, it is reported that PNG is hydrolyzed in alkaline and acidic solution, and the results obtained by this method may not be so accurate because of the low concentration of procaine in parenteral solutions. Another possible method using polarimeter is also unsuitable, because the precise estimation of optical rotation of PNG is complicated by the fact that mutarotation and hydrolysis occur concurrently and the change of optical rotation is only about 0.5° in 20-cm. tube.

Lehman and other investigators[5~10] estimated minute amount of tertiary amines in plasma and urine by the following methods, which are based on the phenomena that strong alkaline organic base forms a complex with acidic dyes, such as sulfophthalein

* Hongo, Tokyo (池田 憲).

1) F. Weygand : Ber., **72**, 163(1939).
2) C. Sannié, M. Vincent : Compt. rend. soc. biol., **142**, 493(1949).
3) J.S. Cannell : J. Pharm. Pharmacol., **3**, 741(1951).
4) T. Kaito : Bull. Hyg. Lab., **72**, 99, 101(1954).
5) R.A. Lehman, T. Aikten : J. Clin. Med., **28**, 787(1942).
6) B.B. Brodie, Udenfriend : J. Biol. Chem., **158**, 705(1945).
7) *Ibid.*, **168**, 335(1947).
8) G. Cromheim, P.A. Ware : J. Pharmacol. Exptl. Therap., **92**, 98(1948).
9) W.A. Dill, A.J. Glzko : J. Biol. Chem., **179**, 395(1949).
10) C.C. Porter, R.H. Silber : *Ibid.*, **182**, 109(1950).

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or methyl orange, and the complex compound is extracted quantitatively with a suitable organic solvent at optimum pH. Therefore, organic base can be estimated by the colorimetry of the extracted dye. Recently, Horioka[11] examined various combinations of dyes, organic solvents, and pH values of buffer solutions suitable for a base to be estimated and proved that it is possible to estimate a single organic amine selectively from a mixture of amines using appropriate combination of reagents. By such a method, it is expected that the complex between PNG and acidic dye may not be extracted with organic solvent, because PNG contains highly hydrophilic group in its molecule, and procaine may be estimated selectively.

The reagents used in this investigation were bromocresol green (BCG) as an organic dye, ethylene dichloride (EDC) as an organic solvent, and a phthalate buffer solution (pH 5.40), and satisfactory results were obtained as expected. In the procedure, which will be described later, the amount of BCG extracted by EDC was strictly proportional to the concentration of procaine and not affected by the presence of PNG and glucose. However, it is necessary to estimate the amount of PNG hydrolyzed in buffer solution during colorimetric procedure. It takes about ten minutes to extract the complex compound in this procedure and so the amount of PNG hydrolyzed in ten minutes was determined. It was proved that 1.3% of PNG is hydrolyzed in 2 hours in a buffer solution and 2.5% in 4 hours, and that the hydrolysis proceeds unimolecularly. Calculation from these data shows that PNG is hydrolyzed only 0.12% in 10 minutes, so the hydrolysis of PNG during colorimetric procedure can be neglected, comparing the total error of estimation.

Glucose and N-glucoside have structural isomers in solution, i.e. α- and β-pyranoside, α- and β-furanoside, and acyclic structure, and N-glucoside undergoes Amadori rearrangement. There may be complicated equilibria between these structures, but only the amount of combined and uncombined procaine can be estimated by the above method. It was proved that the amount of PNG increased and became stationary after some time. The reaction is considered to be reversible. If it is so, the same amount of PNG must also be obtained in a reverse process, namely, by the hydrolysis of PNG. The equilibrium constant K was determined, both in PGI and reverse process. PGI contains 0.018330 mole/L. (a) of procaine hydrochloride and 0.27753 mole/L. (b) of glucose. The solution of reverse reaction (RRS) was prepared to contain equimolar amount of procaine and glucose, i.e. 0.018330 mole/L. (a) of PNG and 0.25920 mole/L. ($b-a$) of glucose. Equilibrium constant K obtained in PGI and RRS agreed strictly at each temperature. Of course, K varies with temperature. After the equilibrium state was obtained at a definite temperature, reaction temperature was settled at another temperature, and it was proved that equilibrium state transits completely.

K was ascertained in different ratios of procaine and glucose. The effect of addition of hydrochloric acid, which is added to prevent hydrolysis of procaine itself, was observed because PNG is known to be hydrolyzed by acids. The amount of added hydrochloric acid was that described in Danish Pharmacopoeia. The addition of electrolyte may affect the activity of ions of procaine and PNG, so the PGIs which contain NaCl and Na_2SO_4 were prepared and K was determined at 100°.

Beside observations on equilibrium, the velocity of formation and hydrolysis of PNG were investigated.

Theoretical Considerations for Equilibria

Considering the fact that formation and hydrolysis of PNG are opposing reactions, following equations are obtained.[12]

11) M. Horioka : J. Pharm. Soc. Japan, **77**, 200, 206(1957).
12) E. A. Moelwyn-Hughes : "The Kinetics of Reactions in Solution," 2nd Ed., Oxford University Press, London, 40, 160.

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$$\underset{a-x}{\text{Procaine}} + \underset{b-x}{\text{Glucose}} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \underset{x}{\text{PNG}} + \underset{w}{H_2O} \qquad (2)$$

where a = initial concentration of procaine
b = initial concentration of glucose
x = concentration of PNG at any time, t
w = amount of water (actual value is 53.558 mole/L., which is calculated from the specific gravity of the solution. In the presence of a neutral salt, correction is necessary).
k_1 = velocity constant of formation of PNG
k_2 = velocity constant of hydrolysis of PNG

The formation of PNG is a bimolecular reaction and its hydrolysis is considered as a pseudounimolecular reaction, because the amount of water is greatly in excess. The rate of formation of PNG can be represented as follows:

$$\frac{dx}{dt} = k_1(a-x)(b-x) - k_2wx \qquad (3)$$

The equilibrium constant K is represented as follows:

$$K = \frac{wx_e}{(a-x_e)(b-x_e)} = \frac{k_1}{k_2} \qquad (4)$$

where x_e is concentration of PNG at the equilibrium. By substituting (4), Eq. (3) is integrated and k_1 is obtained.

$$k_1 = \frac{1}{t\beta} \ln \frac{x(\alpha-\beta)-2ab}{x(\alpha+\beta)-2ab} \qquad 5)$$

where $\alpha = a+b+\frac{w}{K}$

$\beta = \sqrt{\alpha^2-4ab}$

Therfore, the concentration of PNG at optimal time t can be represented as follows:

$$\frac{x}{a} = \frac{2b}{\beta}\left[\frac{\alpha}{\beta} + \coth \ln\left\{\frac{k_1\beta t}{2}\right\}\right]^{-1} \qquad (6)$$

When the concentration of PNG is already m at the starting time of the reaction, k_1 is represented as follows:

$$k_1 = \frac{1}{t\beta} \ln \frac{x(2m-\alpha+\beta)+(2ab-m\alpha-m\beta)}{x(2m-\alpha-\beta)+(2ab-m\alpha+m\beta)} \qquad (7)$$

and after an optinal time t, the concentration of PNG will be represented as follows:

$$x = m+2(ab+m^2-m\alpha)\left[\alpha-2m+\beta \coth \ln\left\{\frac{k_1\beta t}{2}\right\}\right]^{-1} \qquad (8)$$

After sterilization, in which PNG was formed m, the values of k_1 and x are obtained by Eqs. (7) and (8), respectively. The change in RRS can be represented by substitution of a for m in Eqs. (7) and (8). The change of K by temperature is known as van't Hoff reaction isochore.

$$\frac{d \ln K}{dt} = \frac{-\Delta H}{RT^2} \qquad (9)$$

where ΔH = heat of reaction
R = gas constant
T = absolute temperature

The relation between $\log K$ and $1/T$ is linear and the heat of reaction is obtained from the slope of this line. The relation between k_1 and temperature is represented by Arrhenius equation:

$$\frac{d \ln k_1}{dT} = \frac{-E_1}{RT^2} \qquad (10)$$

where E_1 = activation energy of formation of PNG which can be obtained as described above. The relation between activation energies and heat of reaction is presented

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as follows:

$$\Delta H = E_1 - E_2$$

where E_2 = activation energy of hydrolysis of PNG

Experimental

Synthesis of PNG—PNG was synthesized by Cannell's method.[3] m.p. 134~137°. *Anal.* Calcd. for $C_{19}H_{30}O_7N_2 \cdot HCl \cdot H_2O$: C, 50.4; H, 7.34; N, 6.2. Found: C, 50.7; H, 7.30; N, 6.0.

Colorimetry

(1) Reagents: All reagents used here were of extra pure grade.

0.1% BCG solution—0.2 g. of BCG was well ground in a glass mortar with 5.72 cc. of 0.05*N* NaOH and diluted to 200 cc. with distilled water,

Phthalate buffer solution (pH 5.40)—35.25 cc. of 0.4*N* NaOH was added to 50 cc. of 0.4*N* potassium hydrogen phthalate solution. Small amount of toluene was added to prevent contamination of microörganisms.

EDC—Washed with alkaline and acidic water and distilled.

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

Standard solutions of procaine hydrochloride—500 mg. of procaine hydrochloride was dissolved in distilled water to make 1000 cc. and diluted to 25, 50, 75, and 100 γ/cc.

(2) Procedure: 20 cc. of EDC is pipetted into a glass-stoppered centrifugal bottle (capacity. about 50 cc.) aud 2 cc. of buffer solution, 1 cc. of BCG solution, and 1 cc. of test solution are added. The bottle is shaken vigorously for 5 mins. and centrifuged. The aqueous layer is taken off by suction, 10 cc. of EDC layer is transfered into another bottle, and 10 cc. of 0.1 *N* NaOH is added to it. The bottle is shaken and centrifuged as above and the colored aqueous layer is submitted to colorimetry with Beckman DU spectrophotometer at 617 mμ. The regression was calculated from 20 points, which were obtained by 5 repetitions at each concentration of standard solution of procaine hydrochloride. The standard error was about 1.0% at 75 γ/cc. The regression line and its confidence limits in 95% are shown in Fig. 1 by a solid line and broken lines, respectively.

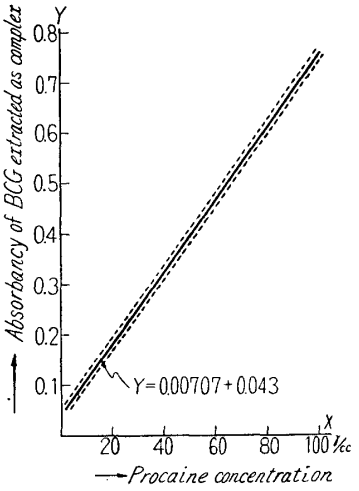

Fig. 1.

Standard Curve

Preparation of samples

(1) PGI—5 g. of procaine hydrochloride and 50 g. of glucose were dissolved in distilled water to make 1000 cc. Then the solution was poured into 5-cc. ampules, and sterilized in boiling water bath for 1 hr. These ampules were divided into 4 groups and stored in thermostats adjusted to 20, 30, and 40°. One group of ampules was boiled further to see the equilbrium at 100°. To examine the changes at 50° and 76°, ampules prepared as above without sterilization were stored in a thermostat adjusted at 50° and in a bath of boiling CCl_4. The thermostat used for 20° was an air bath regulated to within ±2°, and those used for 30, 40, and 50° were water baths with relays, accurate to within ±0.2°.

The ampules were taken out at given intervals from a thermostat, cooled immediately in ice water, 1 cc. of its content was pipetted into a 50-cc. volumetric flask, and diluted with distilled water. Colorimetry was carried out with this diluted solution. pH value of PGI was about 6.0 at the beginning and about 4.0~4.5 at equilibrium.

(2) RRS—8.302 g. of PNG and 46.698 g. of glucose were dissolved in distilled water to make 1000 cc., poured into ampules, and treated as with PGI without sterilization. The pH value of this solution generally agreed with that of PGI.

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(3) Effect of concentration of procaine—1.0% and 0.1(w/v)% solutions of procaine hydrochloride each containning 5(w/v)% of glucose were prepared and value of K was determined.

(4) Effect of hydrochloric acid—5 g. of procaine hydrochloride, 50 g. of glucose, and 10 cc. of 0.1*N* hydrochloric acid were dissolved in distilled water to make 1000 cc. The pH of this solution was about 3.5.

(5) Effects of neutral salts—PGI containing 5(w/v)% of NaCl and 5(w/v)% of Na_2SO_4 respectively were prepared and *K* at 100° was determined.

Results and Discussion

The amounts of free procaine (%) in PGI and RRS at given temperature are shown in Figs. 2, 3, and 4. In these figures solid lines show the calculated curves of Eqs. (6) and (8), substituting the mean values of *k* obtained by Eqs. (5) and (7), and the observed points are dotted. At the initial stage, the amount of PNG formed at higher temperatures is more than those at lower temperatures. At the equilibrium stage, however, higher the temperature, the smaller amount of PNG is formed. These facts show that formation of PNG is an exothermic reaction. The free procaine at the equilibrium in PGI and RRS agreed strictly.

Equilibrium constants *K*, with its 95% confidence limits, and velocity constant k_1 from Eqs. (5) and (7) were calculated and are tabulated in Table I. As seen in this table, the velocity constants obtained in sterilized solutions are greater than those in non-sterilized solutions. This may be explained by the well-known fact that pH value decreases by sterilization.[13] At lower temperatures, calculated velocity constants increased and pH value decreased concurrently. The decrease of pH and increase of

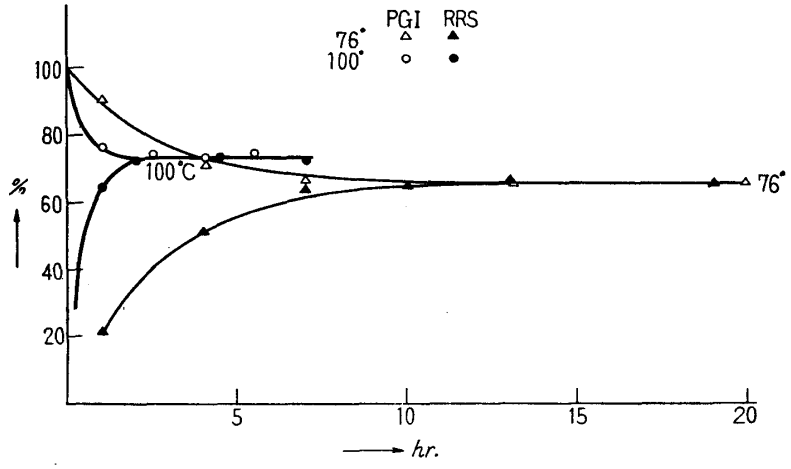

Fig. 2.

Free Procaine Concentration

$\left(\frac{a-x}{a}\times 100\right)$

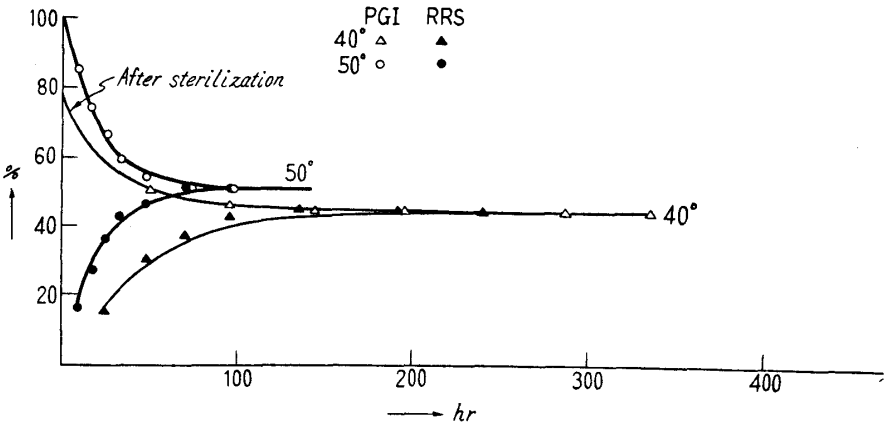

Fig. 3.

Free Procaine Concentration

$\left(\frac{a-x}{a}\times 100\right)$

13) S. Takagi, Y. Maekawa : Japan Analyst, **4**, 34(1955).

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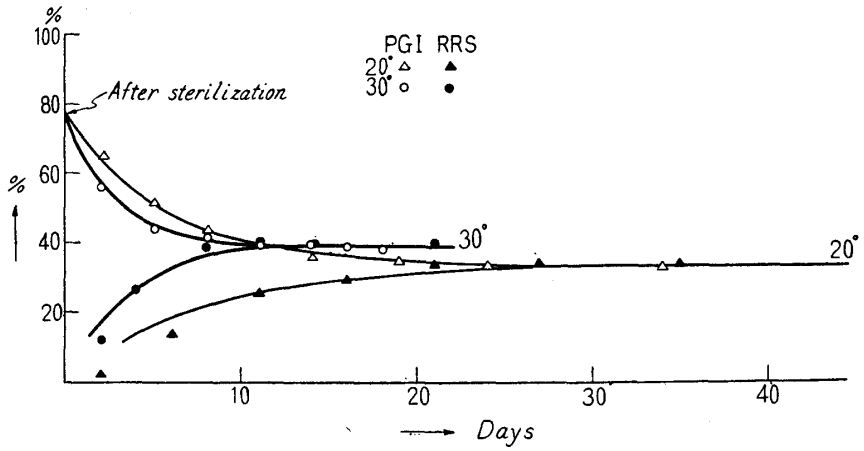

Fig. 4.

Free Procaine Concentration

$\left(\frac{a-x}{a}\times 100\right)$

velocity constant at 50° are shown in Table II. Such tendency can be seen from the difference between the calculated curves and observed values in Figs. 2, 3, and 4.

Table I. Equilibrium Constant (K), and Velocity Constant (k_1, and k_2), at each Temperature

t°C	$K\pm 95\%$ C. L.	k_1(L./mol. hr.) PGI	k_1(L./mol. hr.) RRS	k_2(L./mol. hr.)
20	411 ±49	1.86×10^{-2}*	1.02×10^{-2}	2.48×10^{-5}
30	307 ±24	3.76×10^{-2}*	1.95×10^{-2}	6.35×10^{-5}
40	239 ±15	7.69×10^{-2}*	4.06×10^{-2}	1.70×10^{-4}
50	188 ±15	8.44×10^{-2}	8.74×10^{-2}	4.65×10^{-4}
76	102 ±11	4.20×10^{-1}	4.87×10^{-1}	4.77×10^{-3}
100	71.0± 9.3	2.15	2.00	2.81×10^{-2}

* Obtained in sterilized PGI

Table II. Change in Concentration of PNG, pH, and Velocity Constants caluculated from Eqs. (5) and (7) at 50°

t (hr.)	PGI pH	PGI $x\times10^3$ (mol./L.)	PGI $k_1\times10^2$ (L./mol. hr.)	RRS pH	RRS $x\times10^3$ (mol./L.)	RRS $k_1\times10^2$ (L./mol. hr.)
0	5.80	0		6.10	18.33	
8	5.41	2.93	8.16	5.08	15.40	8.36
16	5.19	5.04	8.44	4.90	13.30	8.55
24	4.94	6.73	8.76	4.38	11.60	9.32
33	4.75	7.88	9.84	4.88	10.45	9.76
∞	4.55	8.89		4.55	8.89	

The transition of equilibria between different temperatures was complete. For instance, the equilibrium constants at 100° of PGI, which at first attained equilibrium states at 30, 40, and 50°, then transferred to 100°, were 70.9, 77.2, and 70.9. The transition of equilibria between another temperature was also proved to be complete. K–Value decreases with the rise of temperature and strictly linear relation between $\log K$ and $1/T$ was obtained over the observed range from 20° to 100°, as shown in Fig. 5, in which open circles show the mean values and upper and lower horizontal lines of the mean values represent their 95% confidence limits. The regression line was represented as $\hat{Y}=1.075X-1.050$, where $\hat{Y}$ is the estimation value of $\log K$ and X is $1/T\times10^3$. The amount of PNG at equilibrium at a given temperature can be calculated from this regression line. It is considered that relatively larger confidence limit at 20° is caused by the less accurate temperature of air bath cooled by ice. The heat of reaction calculated from the slope of regression line was 4.77 Kcal.

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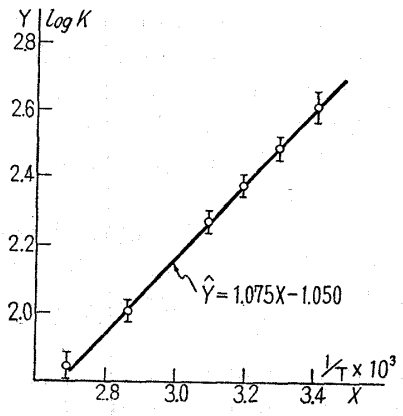

Fig. 5. Relation between $1/T-\log K$

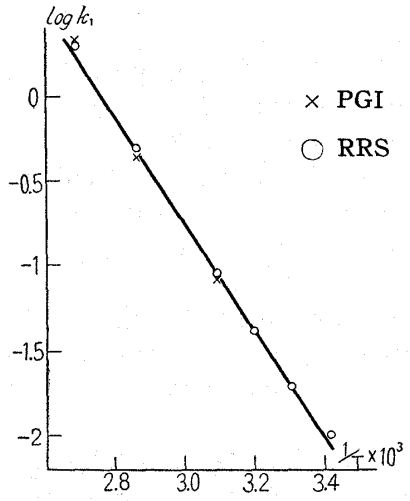

Fig. 6. Relation between $1/T-\log k_1$

The relation between logarithm of the mean values of k_1 and $1/T$ was also linear as shown in Fig. 6. The activation energy of formation of PNG was estimated approximately as 14.0 Kcal., and that of hydrolysis was about 18.8 Kcal. at pH 4~5.

The variation of the K at 100° under various conditions is tabulated in Table III. The proportion of procaine and glucose did not affect K over the observed range. The addition of hydrochloric acid of the amount described in Danish pharmacopoeia also did not affect K. Although the addition of 5(w/v)% of NaCl did not affect K, the addition of 5(w/v)% of Na_2SO_4 seemed to affect K, considering the mean value of K in PGI and its confidence limits.

Table III. Equilibrium Constants under Various Conditions at 100°

Conditions	K
PGI	71.0±9.3
Concn. of procaine, 1.0%	68.0
Concn. of procaine, 0.1%	68.2
PGI-added HCl soln.	68.5*
PGI-added 5% of NaCl	72.6
PGI-added 5% of $NaSO_4$	84.4

* K of this solution at 30° and 76° showed 294 and 100, respectively.

It has been said that the preparations such as PGI have to be prepared by aseptic manipulation and stored in a refrigerator, because heat sterilization and storage at high temperature are considered to increase the amount of PNG. The results obtained in the present study show, however, that a larger amount of PNG forms at lower temperatures at the epuilibrium. After sterlization at 100°, in which 23.5% of procaine combined with glucose in 1 hour, the formation of PNG proceeded further during storage.

Calculations from K show that 1.438% of procaine hydrochloride must be prepared to obtain the PGI with 0.5% of free procaine remaining at the equilibrium at 20°. If PGI is boiled in water for about 1.5 hrs. to obtain the equilibrium at 100° before use of PGI, 0.679% procaine hydrochloride solution must be prepared to obtain 0.5% of free procaine injection.

The author wishes to express thanks and appreciation to Prof. Dr. H. Nogami, Assistant Prof. J. Hasegawa, and Mr. M. Horioka for their kind guidance and encouragement, and to late Mr. M. Wakabayashi for his detailed report on PNG.[14]

14) Unpublished report on synthesis of PNG, its absorbancy of ultraviolet ray, and change of optical rotation of PGI.

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Summary

(1) The decrease of procaine in the aqueous parenteral solution containing 0.5% of procaine hydrochloride and 5% of glucose (PGI) was satisfactorily explained by opposing reactions between formation and hydrolysis of procaine-N-glucoside (PNG).

(2) The formation of PNG was proved to be an exothermic reaction, so that at epuilibrium state, higher the temperature, the less the amount of PNG formed, i.e. 66.5% of procaine was combined at 20° and 26.0% at 100°. The heat of reaction was found to be 4.77 Kcal.

(3) It was found that the activation energies of formation and hydrolysis of PNG were approximately 14.0 and 18.8 Kcal., respectively. There was a tendency that the velocity constant increases with time, which is considered to be caused by the decrease of pH.

(4) From the result obtained, it was concluded that storage of PGI in a refrigerator is not recommended and heat sterilization did not affect the amount of PNG at equilibrium.

(Rcceived December 1, 1956)

U.D.C. 547.677.6

15. Eiji Ochiai, Toshihiko Okamoto, and Mitsutaka Natsume: Syntheses of Alkylphenanthrenes. III.[1]

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In the preceding paper of this series,[1] we reported the syntheses of 1,6,7-substituted alkylphenanthrenes, in which 6 and 7 positions were asymmetrically substituted by methyl, ethyl, or isopropyl group. This paper treats the synthesis of a number of 1- and 6,7-*sym*-substituted alkylphenanthrenes and 1,8-dimethylphenanthrene by the same synthetic method.

One of these compounds, 1,8-dimethylphenanthrene was proved to be identical with a sample[2], obtained by the selenium dehydrogenation reaction of hypognavinol.

1, 8-Dimethyl- and 1-Methyl-8-ethylphenanthrenes

Two synthetic methods for 1,8-dimethyl- and 1-methyl-8-ethylphenanthrenes have already appeared in the literature.[3,4] We present a new, simpler route to these phenanthrenes.

β-(5-Methyl-1,2,3,4-tetrahydro-1-naphthyl)ethanol (II)[5], obtained from 5-methyltetralone-1, was converted to its chloride and reacted with diethyl malonate. After hydrolysis, decarboxylation, and dehydrogenation, γ-(5-methyl-1-naphthyl)butyric acid (III) was obtained which was cyclized to 1-oxo-8-methyl-1,2,3,4-tetrahydrophenanthrene (IV) as the available compound for the next Grignard reaction. Dehydrogenation was effected by heating with palladium-carbon.

The identification of the alkylphenanthrene (m.p. 187~191°) from the alkaloid with

* Hongo, Tokyo (落合英二, 岡本敏彦, 夏目充隆).

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3) Haworth, Mavin, Sheldrick: J. Chem. Soc., **1945**, 454.
4) F.E. King, T.J. King: J. Chem. Soc., **1954**, 1373. cf. P.A. Robins, J. Walker: J. Chem. Soc., **1952**, 1610.
5) L. Ruzicka, K. Hofmann: Helv. Chim. Acta, **22**, 126(1932).

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