skeleton.

Lactate did not give any significant effect in the present experiment, while it was shown in the preceding paper[1] that in the experiment of short duration lactate was as effective as tricarballylate in inhibiting radiostrontium deposition on the skeleton. It is obscure why lactate is ineffective in activating urinary excretion of radiostrontium.

The authors are grateful to Dr. H. Kawakami, University of Chiba, for his kind advice on the histopathological examination of the kidneys, and to Miss S. Iwai for the synthesis of tricarballylic acid. The present series of work was financed by a Grant in Aid for Scientific Research from the Ministry of Education.

Summary

Sodium tricarballylate (8.5 m.mole/kg.) activates the excretion of radioactive strontium injected and reduces its skeketal accumulation. Under a similar condition, sodium lactate (13.5 m.mole/kg.×4) is ineffective. Mechanism of active excretion caused by the injection of tricarballylate was discussed and suggested to be due to its chelating action. Some side effect of tricarballylate injection was observed but it did not produce severe injury.

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UDC 615.782.54-011

9. Jun Hasegawa, Ken Ikeda, and Tai Matsuzawa : Studies on Decomposition and Stabilization of Drugs in Solution. I. Chemical Kinetic Studies on Aqueous Solution of Phenobarbital.

(*Pharmaceutical Institute, Medical Faculty, University of Tokyo**)

The unstability of phenobarbital in aqueous solution has been discussed by many investigators, and several studies[1~6] on its degradation and stabilization have been reported. According to these studies, the decomposition of phenobarbital depends on the pH value and temperature of the solution and it was found that the reaction is accelerated very much in the region of high pH value. Many experimental results were obtained from these reports, but theoretical consideration on the degradation has not been published.

The following study was carried out in order to elucidate the mechanism of the reaction of phenobarbital in aqueous solution from the standpoint of chemical kinetics in an accelerated condition.

Theoretical Consideration of the Reaction

The degradation of phenobarbital in alkaline solution, according to Husa,[2] is as shown in Chart 1.

* Hongo, Tokyo (長谷川淳, 池田 憲, 松沢 兌).

1) Leo Nielsen : Dansk. Tids. Farm., **7**, 137(1933)(C. A., **27**, 5146(1933)).
2) W. J. Husa, *et al.* : J. Am. Pharm. Assoc., **33**, 217(1944).
3) G. C. Walker, *et al.* : Canad. Med. Assoc. J., **71**, 8(1954)(C. A., **48**, 4115(1954)).
4) W. J. O. Reilly : J. Pharm. Pharmacol., **6**, 253(1954).
5) Myra Roberts : Australarian J. Pharm., (C. A., **50**, 8991(1956)).
6) Nelge Nuppenau : Dansk. Tids. Farm., **28**, 194, 261(1954).

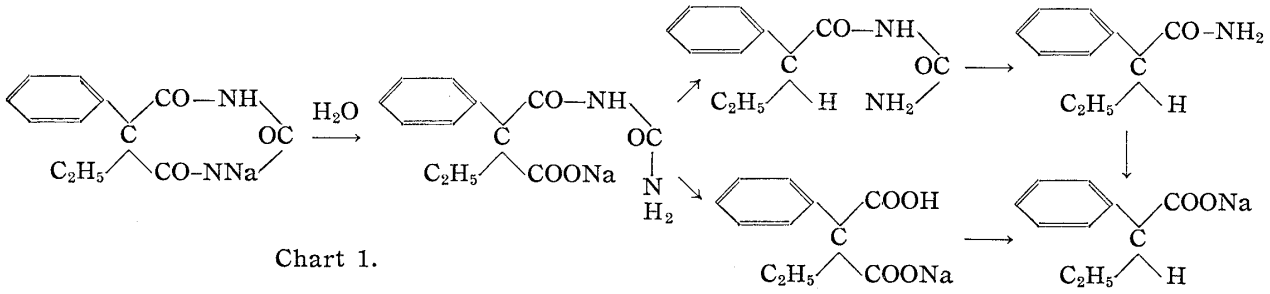

Chart 1.

After considering these formulae and published reports,[7~10] and also our experimental results, we present the theoretical formulae shown in Chart 2. The reaction between pH 6.0~10.5 was examined from the standpoint of the practical and physiological conditions.

At the first step of the reaction, the six-membered ring of phenobarbital is separated by the following two ways, and it reacts as a molecule in (A) and in ionized form in (B).

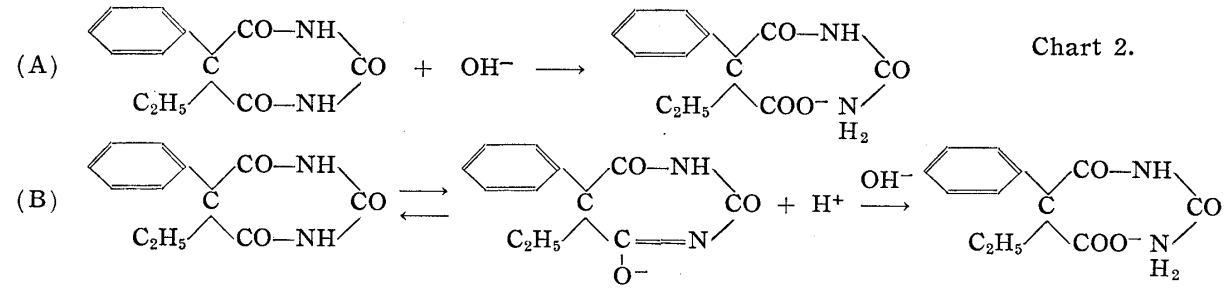

Chart 2.

This reaction may be considered in the same manner as the catalytic hydrolysis of esters by a hydroxyl ion.

Velocity constant in (A).....k_1

Velocity constant in (B).....k_2

The amount of phenobarbital decomposed after any time t, is represented as x_1 and x_2 in the reactions (A) and (B), from which the following equation (1) and (2) are derived.

$$[Ph\cdot H]_t+[Ph^-]_t=C_0-(x_1+x_2) \qquad (1)$$

where $[Ph\cdot H]_t$ is the concentration of molecular phenobarbital after time t, $[Ph^-]_t$ the concentration of ionized phenobarbital after time t, and C_0 the initial concentration of phenobarbital.

$$\frac{[Ph^-]_t}{[Ph\cdot H]_t}=\frac{K_A}{C_H} \qquad (2)$$

where K_A is the dissociation constant of phenobarbital and C_H the concentration of hydrogen ion. From these relationships, we obtain the following equations (3) and (4) for the reactions (A) and (B).

$$\frac{dx_1}{dt}=k_1C_{OH}[Ph\cdot H]_t=k_1C_{OH}\left\{\frac{C_0-(x_1+x_2)}{1+K_A/C_H}\right\} \qquad (3)$$

$$\frac{dx_2}{dt}=k_2C_{OH}[Ph^-]_t=k_2C_{OH}\left\{\frac{C_0-(x_1+x_2)}{1+C_H/K_A}\right\} \qquad (4)$$

where C_{OH} is the concentration of hydroxyl ion. The overall rate of phenobarbital decomposition in time t may be shown by equation (5) :

$$\frac{d(x_1+x_2)}{dt}=\frac{dx_1}{dt}+\frac{dx_2}{dt} \qquad (5)$$

7) Rolf Brodersen : Trans. Faraday Soc., **43**, 351(1947).
8) L. J. Edwards : *Ibid.*, **46**, 723(1950).
9) T. Higuchi, *et al.* : J. Am. Pharm. Assoc., **39**, 405(1950).
10) L. J. Edwards : Trans. Faraday Soc., **48**, 696(1952).

This equation is changed by interpolation of (3) and (4) to

$$\frac{d(x_1+x_2)}{dt}=\{C_O-(x_1+x_2)\}\left\{\frac{k_1C_{OH}}{1+K_A/C_H}+\frac{k_2C_{OH}}{1+C_H/K_A}\right\} \quad (6)$$

In the solution of a fixed pH value, C_{OH} and C_H are constants, and equations (7), (8), and (9) are obtained.

$$\frac{d(x_1+x_2)}{dt}=k\{C_O-(x_1+x_2)\} \quad (7)$$

$$k=\frac{C_{OH}}{K_A+C_H}\{k_1C_H+K_Ak_2\} \quad (8)$$

$$k=\frac{1}{t}\ln\frac{C_O}{C_O-(x_1+x_2)}=\frac{2.303}{t}\log\frac{C_O}{C_O-(x_1+x_2)} \quad (9)$$

From equation (9), we can consider the decomposition of phenobarbital in the solution with fixed pH value as an apparent unimolecular reaction. From equation (8), we obtain equation (8′).

$$\log k=\log C_{OH}+\log\{k_1C_H+k_2K_A\}-\log(K_A+C_H) \quad (8')$$

This equation (8′) may be written approximately as (10) and (11) respectively in the regions of relatively high and low hydrogen ion concentration.

$$\log k=\log C_{OH}+\log k_1 \quad (C_H\gg K_A) \quad (10)$$

$$\log k=\log C_{OH}+\log k_2 \quad (C_H\ll K_A) \quad (11)$$

Therefore, in these regions, linear relationship may be recongnized between the logarithm of velocity constant and concentration of hydroxyl ion.

Experimental

Determination of Phenobarbital and its pKa—It has been reported that phenobarbital in alkaline solution possesses a characteristic absorption in the ultraviolet region, which may be used for the determination[11~13] of phenobarbital by fixing the pH value of the solution.

A buffer solution of pH 9.0 was used as a vehicle throughout this study and the absorption maximum was found at 240 mμ. In this condition, it was evident that the relationship between the concentration and the extinction determined followed the Lambert-Beer rule, and the result is as follows:

$$y = 0.0405\,x$$

where y is the extinction of 1 cm.-cell at 240 mμ and x is the concentration of phenobarbital in γ/cc.

The absorption of decomposed products can be neglected under this experimental condition. In their study,[14] Fox and co-workers reported that pKa of phenobarbital could be determined by its ultraviolet absorption in different buffer systems. The determination was carried out according to their method and the result obtained is shown in Fig. 1. In the region of pH below 4, absorption maximum can not be found and in a higher region the absorption maximum appeared at 240 mμ, and at much higher region it shifted to 254 mμ. The former is due to the univalent ion of phenobarbital and the shift of absorption maximum is caused by the formation of its bivalent ion. The relationship between the molar extinction and pH value at 240 mμ and 254 mμ is shown in Fig. 2. The value of pK_1 can be obtained from the following equation.

$$pK_1=pH-\log\frac{[Ph^-]}{[Ph\cdot H]}f_{Ph^-}=pH-\log\frac{\epsilon_{Ph\cdot H}-\epsilon}{\epsilon-\epsilon_{Ph^-}}+[\text{Correction factor}]$$

where $\epsilon_{Ph\cdot H}$, ϵ_{Ph^-}, and ϵ are the molar extinctions of phenobarbital in undissociated, dissociated, and mixed forms, respectively. In this experiment, the correction factor of ionic strength may be neglected, because the concentration of the buffer solution and phenobarbital used was very small. Therefore, the value of pK_1 agrees with the pH value, which shows the extinction of 1/2 ($\epsilon_{Ph\cdot H}+\epsilon_{Ph^-}$); in other words, at that point where $[Ph^-]=[Ph\cdot H]$. The values were $pK_1=7.42$ and $pK_2=11.87$ from the results obtained and well agreed with the values reported[15~17] (pK_1 7.41,

11) G. V. R. Born: Biochem. J., **44**, 501(1949).
12) Leo R. Goldbaum: Anal. Chem., **24**, 1604(1952).
13) Leland N. Mattson: J. Am. Pharm. Assoc., **43**, 22(1954).
14) Jack J. Fox, *et al.*: Bull. soc. chim. Belges., **61**, 44(1952).
15) Krahl: J. Phys. Chem., **44**, 449(1940).
16) Thomas C. Butles: J. Am. Chem. Soc., **77**, 1486(1955).
17) A. I. Biggs: J. Chem. Soc., **1956**, 2485.

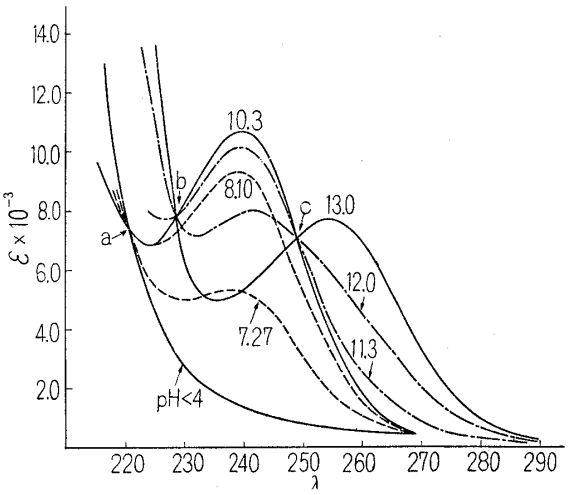

Fig. 1. Absorption Curves of Phenobarbital at Various pH Values
a, b, c=Isosbestic point.

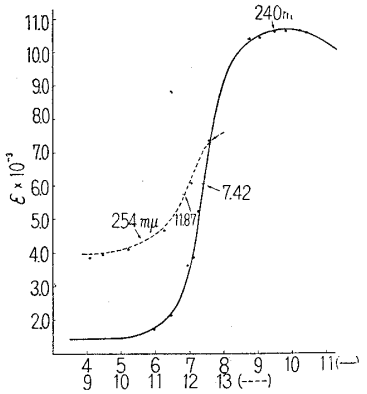

Fig. 2. Molar Extinction at Various pH Values

and pK_2 11.77).

Buffer Solution—A series of buffer solution used in this study were prepared as shown in Table I. The determination of pH value was carried out with the Beckman pH-meter model G.

Experimental Procedure—Phenobarbital (J. P. IV) was recrystallized from dil. EtOH, m.p. 174°. About 100 mg. of the material was dissolved in 200 cc. of the buffer solution to be examined and 5 cc. of the solution was sealed in an ampule. A series of ampules was kept in a thermostatically controlled bath of the desired temperature. When the content of ampule reached the temperature of the bath, one ampule was removed, chilled, 2 cc. of the solution was placed in a volumetric flask, and diluted to 50 cc. with the buffer solution of pH 9.0. The extinction of the diluted solution was determined at 240 mμ and slit width of 0.4 mm. using Unicam Spectrophotometer. This value was considered as the initial concentration, and after a suitable intervals of time for experimental period the sample was taken out and treated in the same manner.

Table I. List of Buffer

Buffer Components (cc./10 cc.)		pH 40°	pH 50°	pH 60°
0.1*M* KH_2PO_4 cc.	0.05*M* $Na_2B_4O_7 \cdot 10\,H_2O$ cc.			
8.77	1.23			6.03
8.30	1.70		6.28	6.28
7.70	2.30		6.48	6.48
7.12	2.88		6.66	6.66
6.58	3.42		6.85	6.85
6.10	3.90		6.98	6.98
5.66	4.34			7.22
0.1*M* HCl cc.	0.05*M* $Na_2B_4O_7 \cdot 10\,H_2O$ cc.			
4.75	5.25	7.52*	7.49*	7.46*
4.50	5.50	7.85*	7.82*	7.79*
4.00	6.00	8.18*	8.13*	8.10*
3.00	7.00	8.45*	8.39*	8.35*
1.50	8.50	8.85*	8.77*	8.70*
0.50	9.50	8.99*	8.91*	8.84*
	10.00	9.08*	8.98*	8.93*
0.1*M* NaOH cc.	0.05*M* $Na_2B_4O_7 \cdot 10\,H_2O$ cc.			
2.00	8.00	9.22*	9.15*	9.07*
3.00	7.00	9.46*	9.35*	9.25*
4.00	6.00	9.66*	9.52*	9.40*
5.00	5.00	10.44*	10.19*	10.00*

* Values corrected by table in H. Yoshimura "pH no Riron to Sokuteiho," Maruzen, Tokyo (1940).

Result and Discussion

The amount of phenobarbital remaining in the buffer solution at 60°, 50°, and 40° after the period of storage is shown in Fig. 3, in which the linear relationship between the log concentration of phenobarbital and time is found. Therefore, the degradation of phenobarbital may be considered as the apparent unimolecular reaction. The velocity constant of the decomposition at each temperature and pH value is shown in Table II.

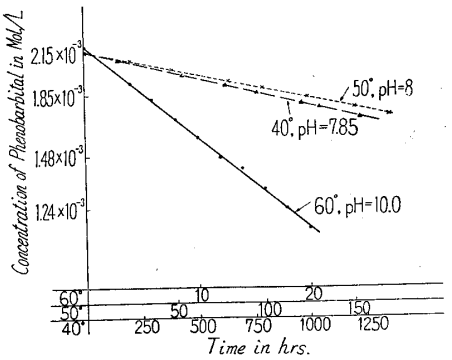

Fig. 3.

First-order Disappearance of Phenobarbital

TABLE II. Velocity Constants at 60°, 50°, and 40°

60°		50°			40°	
pH	$k\times10^4$ hr.$^{-1}$	pH	$k\times10^4$ hr.$^{-1}$ Observed	$k\times10^4$ hr.$^{-1}$ Cal.	pH	$k\times10^4$ hr.$^{-1}$
6.03	1.47	6.28	0.745	0.711	7.52	1.59
6.28	2.35	6.48	1.18	1.09	7.85	1.97
6.48	4.08	6.66	1.58	1.56	8.18	2.34
6.66	6.18	6.85	2.37	2.37	8.45	3.02
6.85	8.15	6.98	3.00	2.93	8.85	4.82
6.98	10.5	7.49	4.97	6.15	8.99	5.64
7.22	12.5	7.82	6.43	8.55	9.08	5.91
7.46	13.7	8.13	8.34	9.27	9.22	7.06
7.79	18.3	8.39	9.68	12.4	9.46	9.01
8.10	20.8	8.77	11.9	15.8	9.66	13.5
8.35	25.9	8.91	13.0	17.2	10.44	49.8
8.70	35.2	8.98	14.7	18.9		
8.84	40.6	9.15	18.6	22.6		
8.93	44.4	9.35	25.7	29.1		
9.07	60.3	9.52	37.7	37.6		
9.25	74.6	10.19	121	133		
9.40	101					
1000.	318					

In this table, the velocity constant was calculated by the following equation :

$$k=\frac{2.303}{t}\log\frac{\epsilon_0}{\epsilon}$$

where ϵ_0 is the initial extinction at $t=0$, and ϵ is the extinction after t hours. Logarithm of the velocity constant is plotted against pH value in Fig. 4, in which the curves I, II, and III are the results at 60°, 50°, and 40°, respectively, Both sides of each curve are straight line with a slope of nearly 1 as expected, and the values of k_1 and k_2 may be caluculated from equations (10) and (11) as described before. A plateau is recognized between acid and alkaline regions.

Using the values of k_1 and k_2, the curve II′ in Fig. 4 was calculated by the following equation :

$$\log k=\log C_{OH}+\log\{3.90\times10^3C_H+7.81\times10\times3.55\times10^{-8}\}-\log\{3.55\times10^{-8}+C_H\}$$

The portion of dotted line in curve III was estimated from curves I and II, because

phenobarbital decomposed very slowly in that region. The values of k_1 and k_2 at each temperature are given in Table Ⅲ.

Curves Ⅱ and Ⅱ′ in Fig. 4 will be examined further. On the acidic side the result obtained agrees well with that calculated, but on the alkaline side a small difference is recognized, and the slope of the straight line is slightly smaller than 1, being about 0.85. The same tendency is recognized at the plateau portion. The difference in this region, however, is 0.1~0.15 at $\log k$, and it may be considered that it is within the limits of error, because k_1 and k_2 are obtained from approximate equations (10) and (11), and the errors from the determination of pH and the correction of temperature on K_A must be added in the calculation of equation (8′).

Besides these errors, there is some possibility of an interaction since reaction (B) is a catalytic one between phenobarbital anion and hydroxyl ion.

Therefore further investigations must be added for the deduction of the reason. Within the range of our study, however, it is considered that the decomposition of phenobarbital may be treated as the hydrolysis catalyzed by a hydroxyl ion, and other catalytic actions, i.e. hydrogen ion and water molecule, are not recognized.

Fig. 5 shows the relation between $\log k_1$, $\log k_2$, and the reciprocal of absolute temperature. The activation energy E_A and the frequency factor A were calcutated using the Arrhenius equation and the results are given in Table Ⅳ. The Arrhenius equation for this reaction obtained therefrom are as follows:

$$k_1 = 1.87 \times 10^{20} \exp\left(\frac{-25600}{RT}\right)$$

$$k_2 = 6.28 \times 10^{18} \exp\left(\frac{-24900}{RT}\right)$$

The velocity constant was examined adding 0.5 mole of sodium chloride to a borax buffer solution at 60°. The pH value changed from 8.94 to 8.85. Then, the velocity constant was 4.17×10^{-3} hour^{-1} and the velocity constant without addition of salt was 4.13×10^{-3} hour^{-1}. Judging from these results, the effect of ionic strength may be neglected in the decomposition of phenobarbital.

Table Ⅲ. Values of k_1 and k_2 at each Temperature

Temp. (°C)	k_1 hr.$^{-1}$ • mol.$^{-1}$	k_2 hr.$^{-1}$ • mol.$^{-1}$
60°	1.29×10^4	2.54×10^2
50°	3.90×10^3	7.81×10
40°	1.01×10^3	2.55×10

Table Ⅳ. Values of Activation Energy (E_A) and Frequency Factor (A)

	k_1	k_2
E_A cal.	25600	24900
A	1.87×10^{20}	6.28×10^{18}

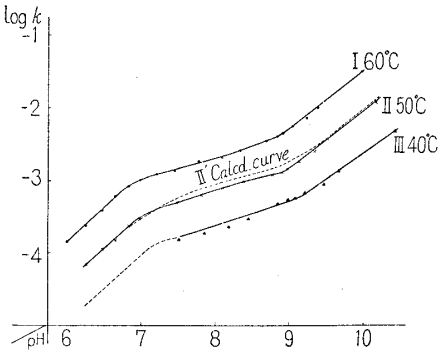

Fig. 4. Overall Velocity Constant for Phenobarbital Hydrolysis as a Function of pH

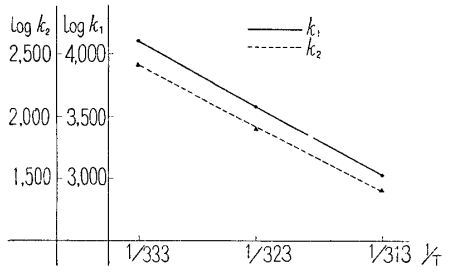

Fig. 5. Temperature Dependence of Reactions (A) and (B)

18) J. F. Bunnett, *et al.*: J. Am. Chem. Soc., **77**, 5051(1955).
19) *Idem.*: *Ibid.* **77**, 5055(1955).

As described above, for the stabilization of phenobarbital, it is necessary that phenobarbital be dissolved in acidic solution, that is, in its molecular form, and not in its ionic from which is decomposed rapidly. The solubility of phenobarbital, however, is far below that for practical use, especially on the acidic side. Water-soluble organic substances, such as propyleneglycol, acetamide, diethyline (glycerin diethyl ether), are commonly added for its solubilization. When a small amount of water is mixed in these preparations, then phenobarbital crystallizes out. In order to overcome these difficulties, it is desirable to prepare a stable and water-soluble complex compound of phenobarbital for its stabilization.

The authors wish to express their thanks and appreciation to Prof. Dr. H. Nogami of University of Tokyo, for his guidance and encouragement throughout this study, and also to Dr. T. Okamoto and Mr. M. Horioka for their discussions.

Summary

The degradative reaction of phenobarbital was investigated over a pH range of 6.0 to 10.5 from the standpoint of chemical kinetics and the following results were obtained.

1) The degradation is an apparent unimolecular reaction at any fixed pH value and is in itself a bimoleculer reaction catalyzed by hydroxyl ion. The presence of any catalysis besides that by hydroxyl ion is not recognized. The relation between $\log k$ and pH is given in Fig. 4.

2) The results obtained well agreed with Eq. (8) which is derived from the postulation described in the theoretical consideration. As seen in Table III, the velocity constant k_1 (molecular form) is larger than k_2 (ionized form), but the decomposition depends on the concentration of hydroxyl ion, and hydrolysis in an alkaline region is very much accelerated.

3) The velocity constant k_2 was slightly smaller than that calculated and further investigation may be necessary for the elucidation of this reason.

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10. Shoji Shibata and Mikio Yamazaki : The Biogenesis of Plant Products. I. The Biogenesis of Rutin.

(*Pharmaceutical Institute, Medical Faculty, University of Tokyo**)

The biological aromatization mechanism has chiefly been investigated by Davis,[1] Sprinson,[2] Tatum,[3] and their co-workers using microörganisms, and the participation of 5-dehydroquinic, 5-dehydroshikimic, and shikimic acids in the biosyntheses of phenyl, *p*-hydroxyphenyl, and 3,4-dihydroxyphenyl derivatives has extensively been elucidated. As for higher plant products, the incorporation of shikimic acid has only been shown by Eberhardt[4] in the lignin formation in sugar cane plant.

An entirely different biogenetical route of aromatization has been suggested for

* Hongo, Tokyo (柴田承二, 山崎幹夫).

1) B.D. Davis : "Amino Acid Metabolism," Johns Hopkins Press, Baltimore, 799(1955).
2) P.R. Srinivasan, D.V. Sprinson, E.B. Kalan, B.D, Davis : J. Biol. Chem., **223**, 913(1956), and earlier papers.
3) E.L. Tatum, S.R. Gross : *Ibid.*, **219**, 797(1956).
4) G. Eberhardt, W.J. Schubert : J. Am. Chem. Soc., **78**, 2835(1956).