It was reported by Maynert and Dawson,[4] that two diasteroisomeric alcohols were isolated as a main metabolite of pentobarbital, but in the case of secobarbital only one of possible diastereoisomeric alcohols was isolated.

Although there must be certain conjugation forms as metabolites, the search for the free forms were only performed in the present study.

As discussed in the previous paper,[1] many metabolites were produced in rabbits administered by Thiamylal, some of which were presumed to be identical with secobarbital and its metabolites by paper chromatography. The studies on the metabolic fate of Thiamylal are continued in the hope of being able to confirm these assumption by utilizing the present study.

The authors are deeply grateful to Mrs. S. Matsuba for the elemental analysis, to Messrs. H. Matsui and K. Hikida for the determination of infrared spectra, and also to the Yoshitomi Pharmaceutical Co. Ltd., for their supply of secobarbital.

Summary

The metabolic fate of secobarbital [5-allyl-5-(1-methylbutyl)barbituric acid] in rabbits were studied and two main metabolites were isolated in addition to the unchanged secobarbital.

The structure of the metabolites were established as 5-allyl-5-(1-methyl-3-carboxypropyl)- and 5-allyl-5-(1-methyl-3-hydroxybutyl)barbituric acid.

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4. Hisashi Nogami,*1 Shoji Awazu,*1 and Yoshio Kanakubo*2 : Studies on Decomposition and Stabilization of Drugs in Solution. XIII. On Sodium Lauryl Sulfate.*3

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The authors[1,2] have been investigating the stabilization of drugs by surface active agents (surfactants), and it was found, in part XII[3], that the hydrolysis of methantheline bromide in a presence of H^+ was accelerated by sodium lauryl sulfate (SLS), and it was ascribed to the attraction of H^+ on the environment of SLS micelle. If this assumption would be correct, the hydrolysis of an ionic surfactant might be influenced by the appearance of micelle as the simpler case than that of methantheline bromide,[3] and the rate of hydrolysis should vary near its critical micelle concentration (CMC). As SLS has been used in this project, it was selected as the first object, and its stability at various pH as well as concentration of SLS was studied.

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*3 Presented at the Kanto Local Meeting of the Pharmaceutical Society of Japan, Tokyo, June, 1961.
1) H. Nogami, *et al.* : This Bulletin, 8, 1136 (1960).
2) *Idem* : *Ibid.*, **10**, 503 (1962).
3) *Idem* : *Ibid.*, **10**, 1158 (1962).

It has already been reported[4~6] that lauryl sulfuric acid was easily hydrolyzed and SLS was also hydrolyzed in acidic solution at a high temperature, but was stable in neutral or alkaline solution. No report on the hydrolysis of SLS was learned in detail.

Experimental

Materials——SLS: A commercial product of Nikko Co., Ltd., recrystallized from EtOH after extraction of higher alcohols with Et_2O.

HCl, H_2SO_4, $CHCl_3$, NaCl, LiOH, and Methylene blue were of G.R.

Na_2SO_4 was of E.P.

Sudan III and Rhodamine 6G were commercial products.

Determination of SLS——SLS was determined by the colorimetric method[7] using Methylene blue. Two cc. of 0.006% Methylene blue and 5% Na_2SO_4 in H_2SO_4*4 solution (pH 1), 8 cc. of H_2SO_4 solution (pH 1), 2 cc. of SLS solution (0.02~0.20 m*M*), and 20 cc. of $CHCl_3$ were pipetted into an about 50 cc. glass-stoppered centrifugal bottle which was shaken for 10 min. by a shaking apparatus and then centrifuged for 5 min. The $CHCl_3$ layer was assayed spectrophotometrically at 660 mμ with a Hitachi EPU-II spectrophotometer. The linear relationship was found between absorbancy and concentration in the limit of 0.02 to 0.20 m*M* of SLS (standard curve).

Procedure——Accurately weighed amount of SLS and 10 cc. of 1*N* or 0.1*N* HCl were placed in a 100 cc. volumetric flask and made to 100 cc. with dist. H_2O. The volumetric flask was immersed in a thermostatically controlled water bath maintained at 70°±0.1°. Samples (2 cc.) were taken from the flask at given intervals, diluted with dist. H_2O (0.02~0.20 m*M* of SLS) and determined as same as the standard curve. Lauryl alcohol produced by the hydrolysis did not interfere the colorimetry.

Solubilization Method with Dye (Sudan III)——Accurately weighed amount of SLS (0.6~10 m*M*) and 1 cc. or 10 cc. of 1*M* NaCl solution were placed in a 100 cc. volumetric flask and made to 100 cc. with dist. H_2O to which Sudan III was added. The flask was immersed in a thermostatically controlled water bath maintained at 70°±0.1°, and was stirred for 2 hr. Samples (5 cc.) were taken by a pipette fitted with a glass filter and 5 cc. of EtOH was added. The solubilization of dye was assayed spectrophotometrically at 520 mμ with a Hitachi EPU-II spectrophotometer. Dist. H_2O was used as a standard. It was learned that 2 hr's., stirring was enough for the solubilization, because the amount of solubilized Sudan III was coincided, even if the stirring period is more than 2 hr. with an experimental error. The precipitation of dye caused by the change of temperature was eliminated by the addition of EtOH.

Fluorophotometric Method——Rhodamine 6G was added to 0.1*M* or 0.01*M* NaCl solution of SLS and 0.1*N* or 0.01*N* HCl solution of SLS (Rhodamine 6G 0.01*M*, and SLS 0.1~10 m*M*). Regarding that the intensity of fluorescence in either NaCl or HCl solution of 10 m*M* SLS was 100 at room temperature respectively, samples were assayed fluorophotometrically at 560 mμ with a Hitachi EPU-II spectrophotometer.

Results and Discussion

1. Hydolysis of SLS in Acidic Solution

Hydrolysis——The results obtained from the experiment with 10 m*M* SLS solution (0.01*N* HCl) during 5 days was illustrated in Fig. 1. The linear relationship between the logarism of SLS concentration and time was recognized at the initial stage, but later the curve descended gradually. It may be considered that SLS is hydrolyzed in the following equation:

$$C_{12}H_{25}OSO_3Na + H_2O \longrightarrow C_{12}H_{25}OH + NaHSO_4$$

*4 In the original Mukerjee's method, HCl was used. But it was found that Methylene blue in a H_2SO_4 solution was less transferrable to $CHCl_3$ layer in the standard, so H_2SO_4 was used.

4) E.W. Maurer, *et al.*: J. Am. Oil Chemists' Soc., **37**, 34 (1960).
5) R.R. Read, W.G. Fredell: Drug & Cosmetic Ind., **84**, 178 (1959).
6) M. Aoki, *et al.*: Yakugaku Zasshi, **80**, 1749 (1960).
7) P. Mukerjee: Anal. Chem., **28**, 870 (1956).

As lauryl alcohol is slightly soluble in water and is solubilized by the presence of SLS micelle, consequently, the state of the solution is varied, therefore it may be considered that the hydrolysis did not follow the pseudo-first order course (Fig. 1).

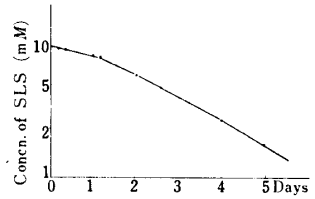

Fig. 1. Hydrolysis of SLS in 0.01NHCl Solution at 70°

The hydrolysis of SLS in various concentrations were illustrated in Figs. 2 and 3. The smaller the initial concentration of SLS, the slower the rate of reaction became in the hydrolysis. The rate of reaction (k) calculated from the initial linear region with the usual equation, assuming the pseudo-first order reaction, was given in Tables I and II.

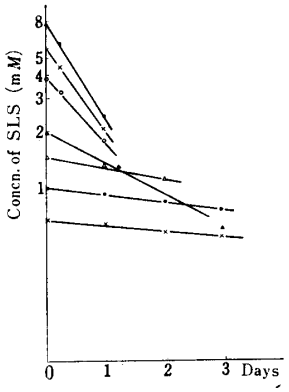

Fig. 2. Hydrolysis of SLS in 0.1NHCl Solution at 70°

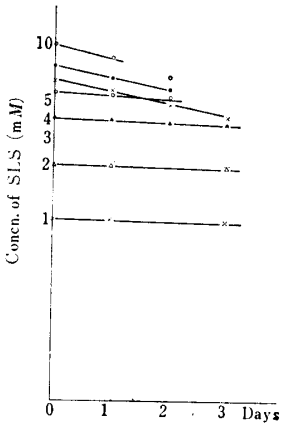

Fig. 3. Hydrolysis of SLS in 0.01NHCl Solution at 70°

Table I. (0.1NHCl)

SLS Concn. (mM)	k(hr^{-1})	$\frac{\Delta k}{\Delta c}$
17.40	0.0383	
		0.0002
7.49	0.0362	
		0.0018
5.53	0.0326	
		0.0033
3.73	0.0266	
		0.0079
1.89	0.0119	
		0.0170
1.39	0.0034	
		0.0032
0.92	0.0019	
		0.0015
0.59	0.0014	

Table II. (0.01NHCl)

SLS Concn. (mM)	k(hr^{-1})	$\frac{\Delta k}{\Delta c}$
9.75	0.0075	
		0.00004
7.53	0.0074	
		0.0031
6.03	0.0027	
		0.0027
5.30	0.0007	
		0.0001
3.91	0.0006	
		−0.0002
2.19	0.0010	
		0.0005
1.07	0.0004	

$\Delta k = k_{i+1} - k_i$ $\Delta c = c_{i+1} - c_i$

k_i : The rate constants where the concentration of SLS is c_i.

The relationship between the rate of reaction and SLS concentration was shown in Fig. 4. The rate of reaction was increased strikingly between 1.39 and 1.89 mM in 0.1NHCl solution and between 6.03 and 7.53 mM in 0.01NHCl solution, respectively.

As already mentioned, if micelles were participating in the hydrolysis, rate values were recognized to be related with the CMC. Therefore, it was necessary to determine the CMC at 70°, which was determined by the follow way.

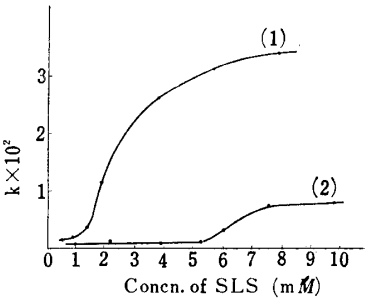

Fig. 4. Relationship between the Rate of Reaction and SLS Concentration in HCl Solution at 70°

(1) 0.1*N* HCl Solution
(2) 0.01*N* HCl Solution

Solubilization Method with Dye at 70°——The solubilization curves of dye in 0.1*M* and 0.01*M* NaCl solution of SLS were shown in Fig. 5 (Absorbancy of solubilized Sudan III-SLS concentration). As the break point where the solubilization of dye increased showed the CMC, it was approximately coincident by this method with the point where the rate of hydrolysis increased.

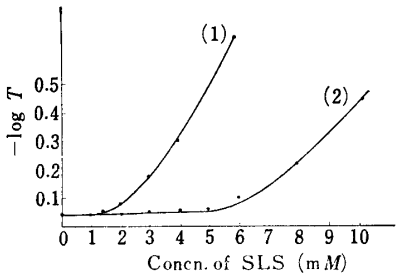

Fig. 5. Solubilization Curve of Dye to NaCl Solution of SLS at 70°

(1) 0.1*M* NaCl Solution
(2) 0.01*M* NaCl Solution

Sodium chloride was used instead of hydrochloric acid in this method, to prevent*5 hydrolysis by acid at a high temperature. The reason is that, according to Corrin and Harkins,[8] the CMC depends on the total gegen-ion concentration and is indifferent from the kind of the ions.

Fluorophotometric Method at Room Temperature——In order to ascertain whether the CMC would be similar in sodium chloride or hydrochloric acid solution at an equimolar concentration, this experiment was carried out. The CMC was obtained in both

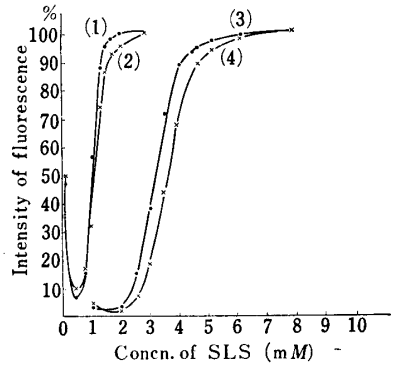

Fig. 6. Intensity of Fluorescence of SLS in NaCl or HCl Solution at Room Temperature

(1) 0.1*N* HCl Solution
(2) 0.1*M* NaCl Solution
(3) 0.01*N* HCl Solution
(4) 0.01*M* NaCl Solution

*5 The hydrolysis of SLS both in water and aqueous solution of 0.1*M* NaCl at 70° were as follows.

	Dist. H_2O	0.1*M* NaCl
Initial Concn. (m*M*)	8.27	8.11
Final Concn.	8.19 (after 20 days)	7.98 (after 10 days)
Initial pH	5.27	5.02
Final pH	3.78 (after 20 days)	3.84 (after 10 days)

Very little hydrolysis were observed.

8) M. L. Corrin, W. D. Harkins: J. Am. Chem. Soc., **69**, 683 (1947).

solutions at room temperature by the fluorophotometry, in order to prevent the hydrolysis by acid happened at a high temperature. The intensities of fluorescence in sodium chloride and hydrochloric acid solutions of SLS were shown in Fig. 6.

As the middle point of the sudden change in intensity was the CMC, any difference has not been considered between H^+ and Na^+. Therefore, it was found that the CMC in sodium chloride solution could be regarded as similar to that in the equimolar solution of hydrochloric acid expediently.

2. Hydrolysis of SLS in Alkaline Solution

If the acceleration effect of the micelle on the hydrolysis of SLS by H^+ can be ascribed to the accumulation of H^+ on the environment of micelle, owing to the coulombic force, the rate of hydrolysis above the CMC in SLS by OH^- will be smaller than that of below.

The hydrolysis of SLS in 3*N* lithium hydroxide solution was shown in Fig. 7. On the contrary to acidic solution, as expected, the solution of SLS at a low concentration was unstable in this case. As dye was salted out, the CMC of SLS in 3*N* electrolyte solution could not be determined by the solubilization method with dye.

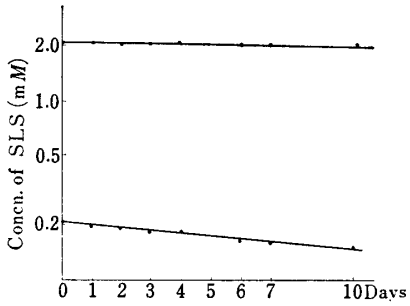

Fig. 7. Hydrolysis of SLS in 3*N* LiOH Solution at 70°

As SLS deposited in a crystalline form at room temperature in 3*N* electrolyte, the CMC could not be determined by the fluorophotometric method neither, however SLS was soluble at 70° and fluorescence was recognized in 2 m*M* solution apparently. Fluorescence was neither recognized in 1, 0.5 nor 0.3 m*M* solution but observed in 0.2 m*M* solution and less concentrations. Consequently, it might be assumed that the micelles were formed in 2 m*M* solution, and not in 0.2 m*M* solution.

On the other hand, the formula[8] of the effect of salts on the CMC of SLS solution was as follows :

$$\log(\mathrm{CMC}) = -0.45774 \log m^+ - 3.2485$$

The value calculated in 0.3 m*M* supports the above assumption.

Surface active agents are amphipathic substances in which hydrophilic and hydrophobic groups exist. It is thought that amphipathic substances are not only used as surface active agents but also as drugs for an other object. When the stabilities of these drugs are investigated, the present study suggests that it is necessary to consider the effect of not only H^+ or OH^- concentrations but also concentrations of drugs.

Summarizing this study, the hydrolysis of SLS was found to be influenced by the presence of the micelle and accordingly the mode of the influence was assumed to vary with the catalytic species, due to the attractive or repulsive force between micelle and species. This assumption is well coincident with part XII.

The authors express their thanks to Mr. K. Sato for his technical assistance and to Nikko Co., Ltd. for the supply of SLS.

Summary

1) Sodium lauryl sulfate (SLS) was hydrolysed in acidic solution at a high temperature, and the rate of hydrolysis was dependent on not only H^+ concentration but also SLS concentration. It was recognized that the rate of reaction was increased strikingly in the region of a certain concentration of SLS.

2) When the Critical micelle concentration (CMC) of SLS was determined by solubilization method with dye (Sudan III), it was approximately coincident with region of a certain concentration where the rate of reaction increased strikingly.

3) On the contrary to acidic solution, in alkaline solution, SLS above the CMC was a little more stable than that of below.

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5. Mitsuko Asai and Satoru Kuwada : Application of Chromatography. XLIV.*1 Action of an Enzyme from *Eremothecium ashbyii* on 6-Methyl-7-(2-hydroxy-2-methyl-3-oxobutyl)ribolumazine.

(*Reaearch Laboratories, Takeda Chemical Industries, Ltd.**2)

Plaut[1] reported previously that the application of an enzyme from *Ashbya gossypii* to 6,7-dimethylribolumazine (G-compound) resulted in splitting of a four carbon compound from the pyrazine ring, which underwent condensation with 6,7-dimethylribolumazine to form riboflavin. As described in the preceding paper,*1 the present authors duplicated the experiment using the enzume of *Eremothecium ashbyii* and obtained nearly the same results, but could not give a definite conclusion about the fate of the uracil compound formed by the removal of the four-carbon compound and the formation mechanism of 6-methyl-7-hydroxyribolumazine (V-compound) produced simultaneously in a large quantity.

Birch and Moye[2] previously condensed the diaminouracil (I; R=H) with the aldol compound[3] II, which is a dimer of diacetyl, at acid pH and converted the product III (R=H) into lumichrome by ring closure with alkali. Later, they also applied this reaction to 4-methylamino-5-aminouracil (I; R=CH_3) and converted the condensation product III (R=CH_3) into lumiflavin by treating with polyphosphoric acid.[4] They also synthesized 6,7,8-trimethyllumazine (IV) and allowed the product to react with diacetyl, but, as the formation of lumiflavin was not observed, they thought that a condensation between the diaminouracil compound and the aldol compound may be more reasonable as the pathway in the biosynthesis of riboflavin than the route which passes through 6,7-dimethylribolumazine.

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*1 Part XLIII : This Bulletin, **9**, 503 (1961).

*2 Juso-nishino-cho, Higashiyodogawa-ku, Osaka (浅井満子, 桑田 智).

*3 Cresswell and Wood (loc. cit.) named this compound 7(2-hydroxy-2-methyl-3-oxobutyl)-6-methyl-8-ribityllumazine.

1) G. W. E. Plaut : J. Biol. Chem., **235**, PC 41 (1960).
2) A. J. Birch, C. J. Moye : J. Chem. Soc., **1957**, 412.
3) O. Diels, W. M. Blanchard, H. v. d. Heyden : Ber., **47**, 2359 (1914).
4) A. J. Birch, C. J. Moye : J. Chem. Soc., **1958**, 2622.