[lit.[3] $[\alpha]_D^{23}$ −82.8°(c=1.1, H_2O)]. For the the paper chromatography of the synthetic bradykinin, six different solvent systems wére employed : Partridge system[16] : BuOH-pyridine-AcOH-H_2O (30:20:6 : 24)[17]; pyridine-BuOH-H_2O(1:1:1)[20]; C_6H_6-BuOH-pyridine-H_2O(1:5:3:3)[21] BuOH saturated with 3% NH_4OH[21]; MeCOEt-pyridine-H_2O(6.5:1.5:2.0).[2] The corresponding Rf values obtained were 0.35; 0.44; 0.49; 0.14; 0.15; 0.073. The spots were developed with ninhydrin and Sakaguchi reagents and in all caces only single spots were obtained. Amino acid ratios in the acid hydrolysate : Arg 2.05, Pro 2.90, Gly 0.91, Ser 0.92.

The authors thank Prof. T. Kameyama, and his co-workers of this college for biological assay, and the staffs of Central Analysis Laboratory, Department of Chemistry, Faculty of Sciences, University of Tohoku, for elemental analysis. They also thank Dr. E. D. Nicolaides, Research Division, Parke Davis & Co. for kind gift of an authentic sample of synthetic bradykinin.

Summary

An alternate synthesis of bradykinin and the synthesis of three analogs of bradykinin is described, in which 4 glycine has been substituted with L-leucine and 6 L-serine has been substituted with O-acetyl-L-serine. The biological activity of three analogs were compared with that of bradykinin.

(Received November 30, 1965)

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20) R. I. Morrison : Biochem. J., **35**, 474 (1953).
21) E. D. Nicolaides, H. A. de Wald : J. Org. Chem., **26**, 3872 (1961).

[Chem. Pharm. Bull. 14(3) 225~232 (1966)]

UDC 615.412.1-011

33. Hisashi Nogami, Tsuneji Nagai,*1 Takao Kasai, and Toshio Kajima*2 : Studies on Powdered Preparations. XVI.*3 Aging of Dried Aluminum Hydroxide Gel in Aqueous Ammonia.*4

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*Chugai Pharmaceutical Co., Ltd.**2)

Generally, an aging of aluminum hydroxide gel is influenced by the surrounding atmosphere and many studies have been reported on this matter. Especially, the crystallization by aging proceeds very rapidly under the condition of a higher pH and on this fact many investigations have been carried out as one of the important factors in manufacturing process. Marboe and Bentur[1] examined the suitable pH range for obtaining the amorphous substance by the reaction between aluminum salts and aqueous ammonia.

In the previous paper,*3 the aging of dried aluminum hydroxide gel (DAHG) J. P. on keep-standing under various relative humidities or in water was investigated, and the neutralizing rate was found to be accelerated temporarily in the process of aging, the final products on aging being supposed to be a mixture of bayerite and gibbsite. It was suggested that the transitory acceleration of the neutralizing rate in the aging

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*1 Hongo, Tokyo (野上 寿, 永井恒司).
*2 Ukima-machi, Kita-ku, Tokyo (葛西敬夫, 梶間俊男).
*3 Part XV. H. Nogami, T. Nagai, T. Kasai, T. Kajima : This Bulletin, **14**, 159 (1966).
*4 Presented at the Kanto Branch Meeting of Pharmaceutical Society of Japan, Tokyo, June 1964.
1) E. C. Marboe, S. Bentur : Silicates Industrials, **26**, 389 (1961).

process might have a profound relationship with the structural change from amorphous to crystalline state.

In this study, the test samples of commercial DAHG were kept in aqueous ammonia of various concentrations at 30° and aging process were observed by the measurements of neutralizing rate, X-ray powder diffraction, and infrared absorption spectra, paying attention to the structural change and crystallizing rate in the aging process. Aqueous ammonia was employed because such experiments as by Marboe and Bentur[1] described above had been reported, and because it was conveniently used for preparing a solution of appropriate high pH and was also easily eliminated from the aged samples for the measurements.

Experimental

Sample——The commercial DAHG J.P. used was the same as the sample G–B in the previous paper,[*3] being amorphous when examined by X-ray diffraction methods. It was dried under the reduced pressure of 10^{-2} to 10^{-3} mm. Hg for 24 hr. for experimental use.

Procedure for Aging of DAHG——Various concentrations of aqueous ammonia, 3.5%, 7.0%, 14%, 28%, were prepared from the purest grade reagent. 600 mg. of DAHG was weighed precisely in a small beaker of 4 ml. capacity and to this 2 ml. of aqueous ammonia prepared at a given concentration was added. The aqueous ammonia should be warmed at 30° prior to the addition. The small beaker was put in the desiccator containing the respective concentration of aqueous ammonia. This desiccator was placed in a vessel of stainless steel, which was immersed in a constant temperature bath at 30° for a certain period of time. These apparatus were the same as described in the previous paper.[*3] After being kept standing, the aged sample was separated rapidly over filter by suction and washed well with distilled water until the filtrate did not show any change with the BTB test paper. They were dried over P_2O_5 in desiccator under milder reduced pressure for 24 hr.

Measuring Articles——Measurements of neutralizing rate, X-ray powder diffraction and IR absorption spectrum were carried out, according to the same method as described in the previous paper.[*3] Growth rate of the crystals was calculated from a change of the integral intensity measured on the magnified record of an X-ray diffraction peak around 18° of 2θ value.

Results and Discussion

Neutralizing Rate of the Aged DAHG in 7% and 14% Aqueous Ammonia

The change of neutralizing rate of DAHG, aged in aqueous ammonia was found to be much faster than that aged under various relative humidities or in water and

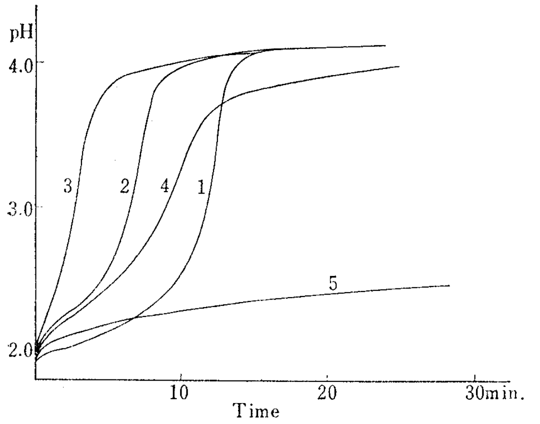

Fig. 1. Reaction between DAHG (Aged in 7% Aqueous Ammonia at 30°) and Hydrochloric Acid at 38° under 600 mg. of Test Sample and 200 ml. of 0.015 N Acid

1 : untreated sample
2 : aged for 5 hours
3 : aged for 15 hours
4 : aged for 24 hours
5 : aged for 48 hours

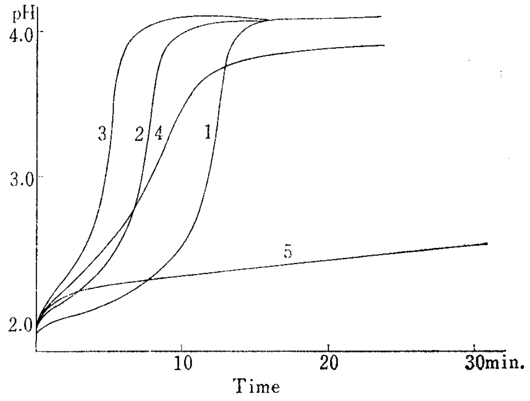

Fig. 2. Reaction between DAHG (Aged in 14% Aqueous Ammonia at 30°) and Hydrochloric Acid at 38° under 600 mg. of Test Sample and 200 ml. of 0.015 N Acid

1 : untreated sample
2 : aged for 1 hour
3 : aged for 2 hours
4 : aged for 15 hours
5 : aged for 24 hours

they lost the neutralizing reactivity after 48 hours in 7% aqueous ammonia and 24 hours in 14% aqueous ammonia. In the process of aging, the change of neutralizing rate observed were pretty similar to the case aged under high relative humidity or in water as was reported in the previous paper. As shown in Fig. 1 and 2, the pH flexional time was found to become shortest around 15 hours' aging in 7% aqueous ammonia and around 2 hours' aging in 14% aqueous ammonia, and as the extention of aging period, the neutralizing rate was decreased. Therefore, in a similar way to the case of water in the previous paper, ammonia was adsorbed on the surface of DAHG and its structure was supposed to be transformed.

As the structure of DAHG is unknown, it is difficult to clarify the reason of the dependence of aging rate on the concentration of aqueous ammonia. However, it may be related to the description by Marboe and Bentur[1] that the aging of aluminum hydroxide gel and the formation of bayerite would be accelerated by the addition of alkali so as to remove protons easily. That is, NH_3 molecule may transform to NH_4^+ ion and protons on DAHG may tend to be decreased, therefore, it is considered that the average protonic density of Al^{3+} inonic groups, surrounded by hydration shell, could be changed. Hence, if the concentration of aqueous ammonia is high, such an action would naturally be strengthened, then the aging rate would be fastened.

Depending on the difference of the concentration of aqueus ammonia, the surface of DAHG undergoes different influence and a domain having different properties is considered to be produced. Therefore, it is expected that the crystallization of growing domain may afford different kinds of crystalline substance, as is confirmed by the results described later.

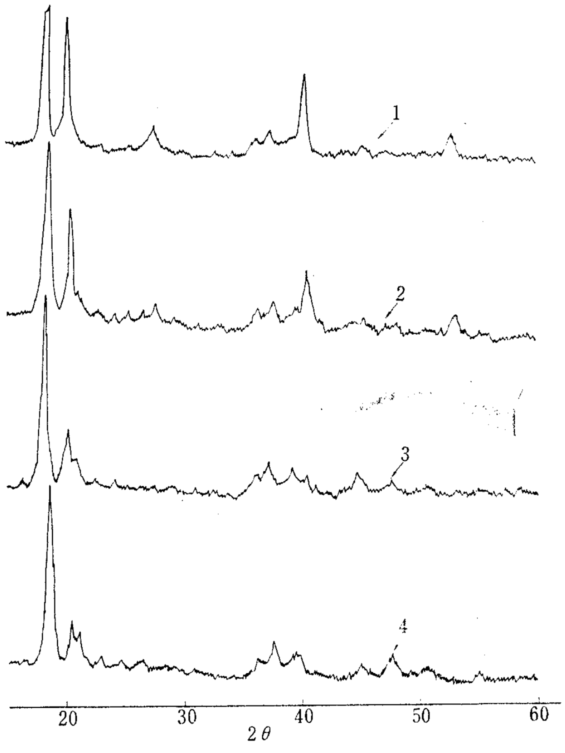

Fig. 3. X-Ray Powder Diffractions of DAHG Aged in Aqueous Ammonia at 30°, by Cu-*Kα* Radiation at a Scanning Speed of 2° per min.

1 : aged in 3.5% aqueous ammonia for 15 days
2 : aged in 7.0% aqueous ammonia for 15 days
3 : aged in 14.0% aqueous ammonia for 15 days
4 : aged in 24.0% aqueous ammonia for 10 days

X-ray Observations of DAHG Aged in Various Concentrations of Aqueous Ammonia

In Fig. 3, X-ray powder diffraction patterns of DAHG aged in aqueous ammonia for 10 or 15 days are shown, indicating clear difference of crystalline substance obtained in different concentration of aqueous ammonia. The sample aged in 3.5% aqueous ammonia was, at first glance, bayerite-like, but when the peak around 18° of 2θ value was recorded at a slacken scanning speed, it was found not to consist of bayerite alone, as is shown in Fig. 4-1. The sample aged in 7% aqueous ammonia, supposing from Figs. 3-2 and 4-2, was considered to be a mixture of bayerite and gibbsite.

The diffraction of the sample aged in 14% aqueous ammonia, as shown in Figs. 3-3 and 4-3, showed to be nearly of a single kind of crystal form, not belonging to that of either bayerite or gibbsite, the peak around 18° of 2θ value being very sharp. 2θ of the peak was calculated to be 18.5°

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2) Part Ⅶ. H. Nogami, T. Nagai : This Bulletin, **10**, 728 (1962).

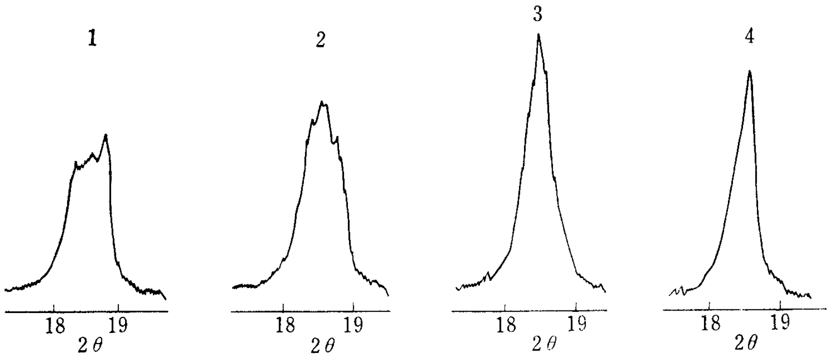

Fig. 4. X-Ray Powder Diffractions of DAHG Aged in Aqueous Ammonia at 30°, by Cu-$K\alpha$ Radiation at a Scanning Speed of 1/2° per min.

1 : aged in 3.5% aqueous ammonia for 15 days
2 : aged in 7.0% aqueous ammonia for 15 days
3 : aged in 14.0% aqueous ammonia for 15 days
4 : aged in 28.0% aqueous ammonia for 10 days

(d=4.79Å), which was found to be similar to that of an alumina trihydrate measured by Nordstrand, *et al.*[3] in 1956. The sample aged in 28% aqueous ammonia, as shown in Figs. 3-4 and 4-4, showed also a similar peak at 18.5° of 2θ to the case of aging in 14% aqueous ammonia, however, a pretty difference was found in the details. In 1958, Papeé *et al.*[4] obtained a similar alumina trihydrate, having the peak at 18.5° of 2θ

Table I. X-Ray Diffraction Data of Nordstrandite

Peak No.[c]	By Papeé, *et al.*		By Nordstrand, *et al.*		In this study	
	d[a]	I[b]	d[a]	I[b]	d[a]	I[b]
1	4.790	FF	4.785	100	4.79	100
2	4.373	f	—	—	—	—
3	4.310	m	4.33	20	4.32	23
4	4.205	〃	4.20	15	4.20	18
5	4.153	〃	4.15	13	—	—
6	3.880	〃	3.89	7	—	—
7	3.600	〃	3.60	7	3.61	6
8	3.462	f	—	—	—	—
—	—	—	—	—	3.38	6
—	—	—	3.18	11	—	—
9	3.022	f	—	—	—	—
10	2.845	〃	—	—	—	—
11	2.706	ff	—	—	—	—
12	2.490	〃	—	—	—	—
13	2.478	m	—	—	—	—
14	2.451	f	—	—	2.45	14
15	2.390	F	2.39	15	2.37	23
16	2.261	〃	2.26	15	2.27	15
—	—	—	—	—	2.24	16
17	2.029	f	—	—	—	—
18	2.013	m	2.01	11	2.00	7
19	1.898	〃	1.89	11	1.90	12
20	1.777	〃	—	—	1.79	7

a) interplanar distance in Å
b) relative intensity FF : intense F : strong m : medium f : faint ff : very faint
c) numbering by Papeé, *et al.*

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3) R.A. van Nordstrand, W.P. Hettinger, C.D. Keith : Nature, **177**, 713 (1956).
4) D. Papeé, R. Tertian, M.R. Biais : Bull. soc. chim. France, **1958**, 1301.

value, by reacting aluminum nitrate solution with aqueous ammonia, but there was some difference of pattern in detail from that obtained by Nordstrand, *et al.* At present, alumina trihydrate having the peak at 18.5° of 2θ is generally called nordstrandite. In Table I, data on the sample aged in 28% aqueous ammonia in this study and those obtained by Nordstrand, *et al.* and by Papeé, *et al.* are compared.

Next, an interesting fact was that the crystallization process by aging was observed in two steps X-ray crystallographically. In Fig. 5, the aging process in 7% aqueous ammonia was recorded with the lapse of time. At the beginning of the aging, a change was observed around 36~42° of 2θ value and it is inclined to increase gradually with the lapse of time. Secondly, after a certain period of time, a change was observed in a portion of 18~20° of 2θ which developed rapidly to the strongest peak with the lapse of time as is shown in Fig. 3-2, accompanying the increase of the diffraction intensity around 36~42° of 2θ. A similar tendency was observed in the case of 14% aqueous ammonia, as shown in Fig. 6. This phenomenon is supposed to have some relation to the change of neutralizing rate.

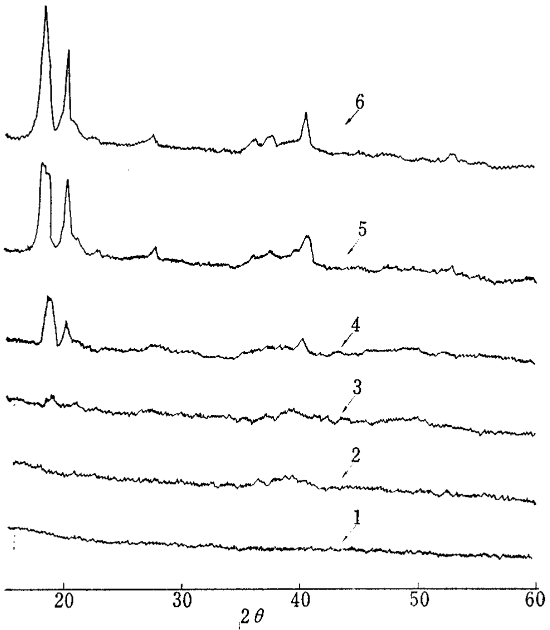

Fig. 5. X-Ray Powder Diffractions of DAHG Aged in 7% Aqueous Ammonia at 30°, by Cu-$K\alpha$ Radiation at a Scanning Speed of 2° per min.

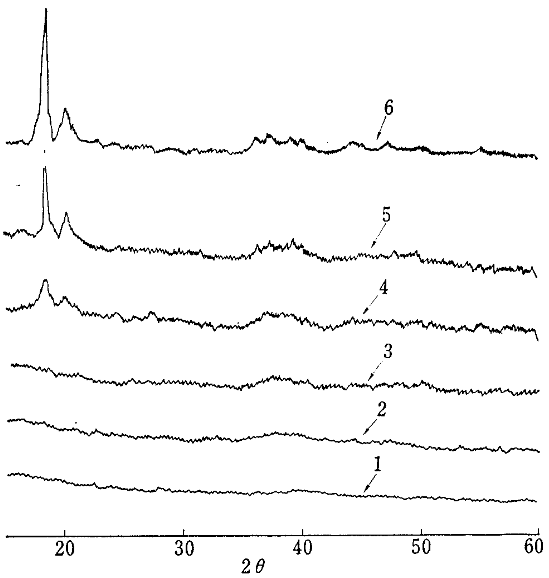

Fig. 6. X-Ray Powder Diffractions of DAHG Aged in 14% Aqueous Ammonia at 30°, by Cu-$K\alpha$ Radiation at a Scanning Speed of 2° per min.

1: aged for 5 hours
2: aged for 15 hours
3: aged for 25 hours
4: aged for 48 hours
5: aged for 72 hours
6: aged for 192 hours

To a certain period of time after the beginning of aging, the neutralizing rate was increased, as mentioned above. While the neutralizing rate was accelerating, *i.e.*, in 15~24 hours on aging in 7% aqueous ammonia and in 10~15 hours on aging in 14% aqueous ammonia, the diffraction around 36~42° of 2θ value appeared and increased gradually. In each case, by the time of appearance of the peak at 18~20°, the neutralizing reactivity was rapidly decreased. 36~42° of 2θ value being 2.5~2.1Å of interplanar distance, the phenomenon observed at an early stage of aging prior to the appearance of the peak at 18~20° of 2θ was explained that a pair of atoms having 2.1~2.5Å atomic distance was arranged orderly, which was considered to be in reactive state with acid. Furthermore, an apearance of the peak at 18~20° of 2θ indicated a real crystallization was proceeded and so the atoms were to be arranged in unreactive state with acid.

Growth Rate of Crystal on Aging of DAHG in Various Concentrations of Aqueous Ammonia

Usually crystallization may be considered in two separate steps, that is, the nucleation and growth.[5] The appearance of the peak at 18～20° of 2θ and its development, as described above, was considered to correspond to the growth period on crystallization, and the fact that at an early stage of aging, a very broad and weak diffraction appeared around 36～42° of 2θ was interpreted to be the induction period. Growth rate of crystal has been studied by many workers,[5] *e.g.*, in the solution by measuring the change of ionic concentration.[6,7] In the present study, the strongest portion of intensity around 18° of 2θ for DAHG aged in various concentrations of aqueous ammonia was recorded at a slacken scanning speed, and the integral intensity was measured by planimeter. These values were plotted against the keep standing period as shown in Fig. 7. The induction period of crystallization was shortened as the concentration became higher, but it was difficult to find an accurate relationship between rate and concentration. However, in the case of 3.5% aqueous ammonia the crystallization was supposed to proceed farther after 15 days and was almost completed by the aging for 15 days in the cases of 7%, 14%, and 28% aqueous ammonia. In the case of 28% aqueous ammonia, the final integral intensity was smaller than those in 7% and 14% aqueous ammonia. This phenomenon was explained by considering that, in the case of less than 14% concentration of aqueous ammonia, as is mentioned before, the aging product was not single but a mixture of bayerite, gibbsite and nordstrandite, and their diffraction might be overlapped each other around 18° of 2θ value, as shown in Fig. 8.

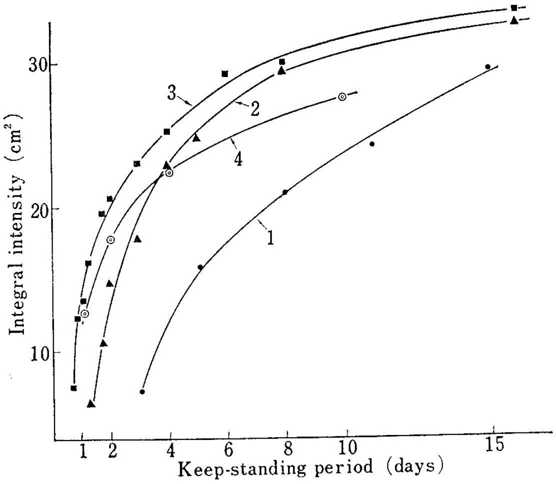

Fig. 7. Crystallization of DAHG in Aqueous Ammonia at 30°, According to the Change of Integral Intensity of the Peak around 18.5° of 2θ Measured by Planimeter

1: in 3.5% aqueous ammonia
2: in 7.0% aqueous ammonia
3: in 14.0% aqueous ammonia
4: in 28.0% aqueous ammonia

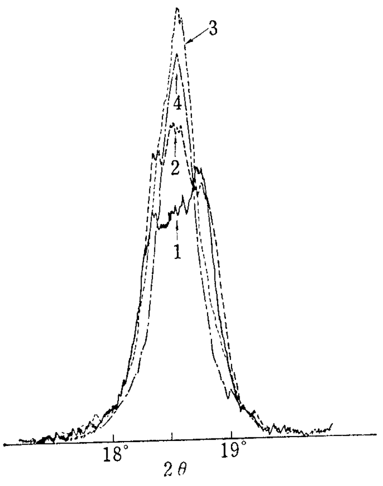

Fig. 8. X-Ray Powder Diffractions of DAHG Aged in Aqueous Ammonia at 30°, by Cu-$K\alpha$ Radiation at a Scanning Speed of 1/2° per min.

1: aged in 3.5% aqueous ammonia for 15 days
2: aged in 7.0% aqueous ammonia for 15 days
3: aged in 14.0% aqueous ammonia for 15 days
4: aged in 28.0% aqueous ammonia for 10 days

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[5] Crystal Growth, Disc. Farad. Soc., **5** (1949); W. E. Gerner: "Chemistry of Solid State" (1955), Butterworth Scientific Pub., London.

[6] R.A. Johnson, J.D. O'Rourke: J. Am. Chem. Soc., **76**, 2124 (1954).

[7] S. Miura, T. Otani: Nippon Kagaku Zasshi, **77**, 517 (1956).

Based upon these facts, judging that the product aged in 28% aqueous ammonia was comparatively of a single kind of crystal form the existing experimental equations[6~8] were employed on Fig. 7-4, *i.e.*,

$$\frac{dx}{dt}=kx^{2/3}(x_\infty-x)^n \tag{1}$$

where x is the integral intensity measured by planimeter at the time t, x_∞ the integral intensity at a time when the crystallization will be finished, and k and n are constants. Equation (1) will be rewritten as,

$$\log\left\{\left(\frac{dx}{dt}\right)\Big/x^{2/3}\right\}=\log k+n\log(x_\infty-x) \tag{2}$$

Estimating a fitting value for x_∞ from Fig. 7-4 and plotting $\log\left\{\left(\frac{dx}{dt}\right)\Big/x^{2/3}\right\}$ against $\log(x_\infty-x)$, a straight line was obtained as in Fig. 9. From this line $n=2.5$ and $k=5.7$ $(\mathrm{cm}^2)^{1/3-n}$/day were given, and the growth rate of crystallization in the case of 28% aqueous ammonia was said to be adaptable to equation (1).

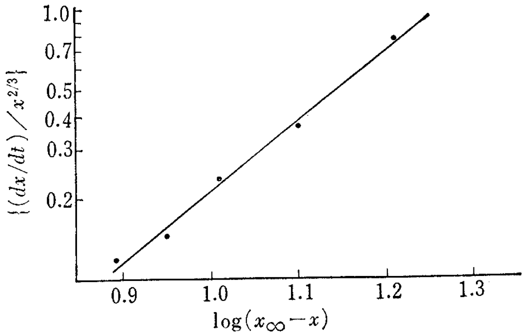

Fig. 9. Crystallization of DAHG in 28% Aqueous Ammonia at 30°, Plotted According to Equation (2)

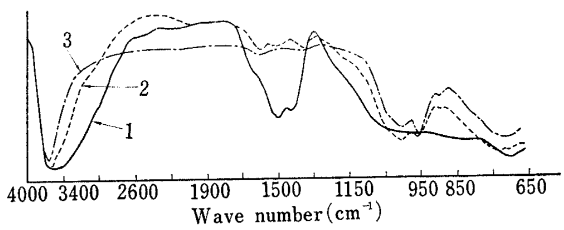

Fig. 10. Infrared Absorption Spectra of DAHG Aged in Aqueous Ammonia at 30°, According to the Potassium Bromide Method

1: untreated sample
2: aged in 7% aqueous ammonia for 6 days
3: aged in 14% aqueous ammonia for 15 days

Infrared Observations of DAHG Aged in Aqueous Ammonia

In the previous paper,*[3] infrared spectra of the samples aged under higher humidity of more than 90% relative humidity at 30° were examined, and as the aging proceeded, a shift of the absorption around 3700~3400 cm^{-1} took place and especially the characteristic band for the aged sample, which lost the neutralizing reactivity, was observed around 1020~960 cm^{-1}.

As it is clear in Fig. 10, on the aging in aqueous ammonia, similar changes to the cases under humidity were recognized at the same positions. But, though the positions of absorption were the same, still slight differences were observed. Especially, the absorption around 3400 cm^{-1} was sharp compared with that of the sample aged under humidity in the previous paper, and it was recognized to be shifted remarkably to 3700 cm^{-1}. This tendency was especially remarkable when the concentration of aqueous ammonia was high. This phenomenon was explained by considering that, by adding ammonia, some change was given to the state of water molecules surrounding Al^{+3} ion in the structure of DAHG and the hydrogen bond was remarkably decreased.

From these results, it seems possible that, as the aging proceeds, the structure of DAHG, in which aluminum ion is bound with such water molecules as are rich in hydrogen bond, tends to change to the orderly arrangement of Al-OH and water coordinated to alumiunm.

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8) K. Takiyama: Bull. Chem. Soc. Japan, **31**, 944 (1958).

This study was supported in part by a Grant in Aid for Fundamental Scientific Research from the Ministry of Education, to which the authors are grateful.

Summary

Commercial DAHG J.P. was kept in various concentrations of aqueous ammonia and the following results were obtained.

1. When the sample was aged in aqueous ammonia, a temporary acceleration of the neutralizing rate was observed at an early stage of the aging process, as was similar to the results obtained in the previous paper.

2. The process of aging was composed of two steps: an increase of the diffraction around 36~42° of 2θ value and an appearance and development of the peak at 18~20° of 2θ. The former is considered to be related with the temporary acceleration of the neutralizing rate.

3. Depending on the concentration of aqueous ammonia, the aging product was different, and especially in the case of 28% aqueous ammonia, it resembled to nordstrandite.

4. The growth rate of crystal on the aging, in the case of 28% aqueous ammonia, was explained by the existing experimental equation, *i.e.*, by equation (1).

5. The infrared absorption around 3400 cm^{-1} was sharp compared with that of the aged sample in various atmosphere under relative humidity in the previous paper, and it was recognized to be shifted remarkably to 3700 cm^{-1}.

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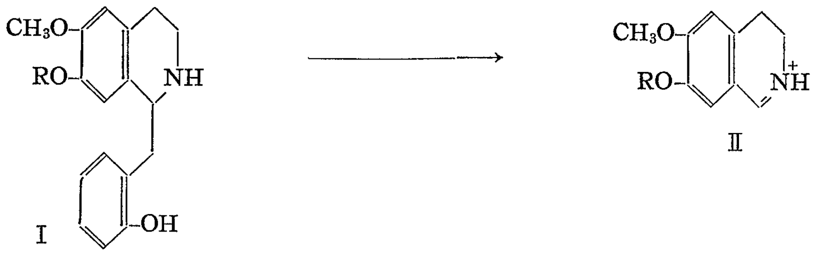
(Chem. Pharm. Bull. 14(3) 232~237 (1966))

UDC 581.19 : 582.675.4 : 547.94

34. **Masao Tomita, Hiroshi Furukawa, Tohru Kikuchi, Akira Kato, and Toshiro Ibuka**: Studies on the Alkaloids of Menispermaceous Plants. CCXX.[*1] Mass Spectra of Benzylisoquinoline Alkaloids.

(*Faculty of Pharmaceutical Sciences, Kyoto University*[*2])

The mass spectrum of 1-(2-hydroxybenzyl)-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (I, R=H), a member of benzylisoquinoline alkaloids, has been examined by Djerassi and his collaborators,[1] who reported that the compound (I, R=H)



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[*1] Part CCXIX. R.H.F. Manske, M. Tomita, K. Fujitani, Y. Okamoto: This Bulletin, **13**, 1476 (1965).

[*2] Sakyo-ku, Kyoto (富田真雄, 古川 宏, 菊池 徹, 加藤 旭, 井深俊郎).

[1] M. Ohashi, J.M. Wilson, H. Budzikiewicz, M. Shamma, W.A. Slusarchyk, C. Djerassi: J. Am. Chem. Soc., **85**, 2807 (1963).