buffer (pH 7.4) were immersed in five ml of various concentration of the drug solution (1×10^{-5}—1×10^{-3} M) dissolved in the same buffer solution. The concentration of free TMU and TEU were determined spectrophotometrically with a Shimadzu UV-200 spectrophotometer at 228 and 226 nm, respectively. That of TBU was determined with a Hitachi double-wavelength spectrophotometer, model 356, using a wavelength pair of 226—280 nm. The data in the form of moles of bound drug per mole of BSA, r, and equilibrium concentration of free drug, c, were analyzed by nonlinear least-squares fitting as described previously.[3]

Fluorescence titration was carried out at room temperature. Two ml of BSA (9.85×10^{-6} M) or BSA (9.60×10^{-6} M) and *p*-toluenesulfonyl-*n*-alkylurea (9.90×10^{-6} M) solution in 1/15 M phosphate buffer (pH 7.4) was titrated with dansylglycine dissolved in methanol. The final concentration of dansylglycine was varied from 5×10^{-7} to 2×10^{-5} M. The fluorescence intensity was measured a 480 nm with excitation at 352 nm, using a Hitachi fluorescence spectrometer, model MPF-3. The binding paramters, n and k_a, of dansylglycine for BSA were 1.027 and 5.316×10^{-5} M^{-1}, respectively.

The pH measurements were carried out with a Hitachi-Horiba pH meter, model M-7, calibrated with standard buffer solutions.

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Microcrystallization Method for Amobarbital and the Physicochemical Properties of the Products[1]

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A method for the microcrystallization of amobarbital was developed. This method consists of the following procedures: (1) dissolution of amobarbital (J.P. IX) in a hot solvent such as a glycol or glycol derivative, (2) pouring this solution into cold water (3°) while stirring to precipitate microcrystals, and (3) collection and drying of the precipitated microcrystals. The crystalline properties of the pulverized crystals of amobarbital were also studied. The degree of pulverization of amobarbital by recrystallization from glycols and their derivatives decreased with increase in the number of carbon atoms in the hydrophobic chain of these solvents.

Examination of pulverized amobarbital was carried out by IR, X-ray diffraction, and DSC–TG, and two kinds of polymorphs were detected.

Keywords——amobarbital; microcrystallization; glycols; glycol derivatives; mean particle diameter; polymorphism

It is generally known that the absorption of drugs is often influenced by their physicochemical properties and dosage form, and it has been reported in many papers that practically insoluble drugs sometimes show high serum concentrations only when administered in a pulverized form.[3–6]

One of the authors (Y. Kato) previously reported higher serum concentration and increased urinary excretion as a result of pulverization of aspirin,[7,8] and the pulverization of aspirin,[9]

1) Microcrystallization Methods for Drugs V. Part IV: Y. Kato, H. Watanabe, N. Maeyama, and K. Kido, *Chem. Pharm. Bull.*, **27**, 366 (1979).
2) Location: *12 Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo, 162, Japan.*
3) J.G. Reinhold, F.J. Phillips, and H.F. Flippin, *Am. J. Med. Sci.*, **210**, 141 (1954).
4) K. Sekiguchi and N. Obi, *Chiml. Pharm. Bull.*, **9**, 866 (1961).
5) J.H. Fincher, J.G. Adams, and H.M. Beal, *J. Pharm. Sci.*, **54**, 704 (1965).
6) J.G. Miller and J.H. Fincher, *J. Pharm. Sci.*, **60**, 1733 (1971).
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9) Y. Kato, S. Togawa, and H. Watanabe, *Yakuzaigaku*, 34, 135 (1974).

phenobarbital,[10] and sulfamethizole[1] has been reported to have similar effects. We report herein some findings on the microcrystallization of amobarbital from glycols and other solvents.

Experimental

Method of Microcrystallization——A solution of 5 g of amobarbital (particle size, 50—80 μm) in 40 ml of a solvent was heated at about 80°, filtered while hot, and the filtrate was poured immediately into about 400 ml of cold water at 3°. This mixture was stirred at 870 rpm for 3 min and the precipitated crystals were collected by suctional filtration. The crystals were washed thoroughly with cold water and dried in a desiccator to obtain finely crystalline amobarbital in about 85% yield. Solvents used for the recrystallization are shown in Table I.

Table I. Solvents used for Recrystallization

Solvent	Number of carbon atoms in hydrophobic chain
Chloroform	
25% Ethanol	
Methanol	
Ether	
Acetone	
Ethylene glycol (E.G.)	
Diethylene glycol (D.E.G.)	
Triethylene glycol (T.E.G.)	
Ethylene glycol monoethyl ether (Cellosolve)	2
Ethylene glycol isopropyl ether (Isopropyl cellosolve)	3
Ethylene glycol monobutyl ether (Butyl cellosolve)	4
Diethylene glycol monomethyl ether (Methyl carbitol)	1
Diethylene glycol monoethyl ether (Carbitol)	2
Diethylene glycol monobutyl ether (Butyl carbitol)	4
Propylene glycol (P.G.)	
Dipropylene glycol (D.P.G.)	
Hexylene glycol (H.G.)	
Propylene glycol monomethyl ether (1-methoxy-2-propanol)	

Materials——Amobarbital of J.P. IX grade was obtained from Nippon Shinyaku Co., Ltd., Kyoto, Japan. The solvents used for the recrystallization were distilled and purified in the conventional way.

Measurement of Particle Diameter——The particle diameters were measured by the sedimentation method (light scanning method) using a PSA-Hitachi model II particle diameter distribution measurement apparatus. Liquid paraffin was used as a dispersion medium for amobarbital (J.P. IX), and a mixture of liquid paraffin and hexane for the microcrystals of amobarbital.

X-Ray Powder Diffraction——A Geigerflex model 2012 (Rigaku-Denki Co., Ltd., Tokyo, Japan) was used (Ni filter, Cu-$K\alpha$ radiation).

Infrared Spectroscopy (IR)——IR spectra were determined with a Hitachi model 225 grating infrared spectrophotometer using the KBr method.

Differential Scanning Calorimetry-Thermogravimetry (DSC-TG)——A Rigaku-Denki DSC-TG (CN.-8085-DI) machine was used, at a heating rate of 10 K/min. N_2 was used as a carrier gas.

Results and Discussion

Volume surface mean diameters measured by the sedimentation method for the microcrystals obtained by recrystallization using various solvents are listed in Table II.

The size of the recrystallized particles was reduced to 1/2 to 1/24 of that of ordinary amobarbital, depending on the solvent used. The crystal forms obtained from each solvent will be described later.

10) Y. Kato and M. Matsuura, *Yakugaku Zasshi*, **98**, 11 (1978).

Table II. Mean Particle Diameter as determined by the Sedimentation Method of Pulverized Amobarbital

Solvent	Crystalline form	Mean particle diameter (μm)	Standard deviation
— (J.P.IX)	I	67.15	23.64
Chloroform	I	5.63	1.93
25% Ethanol	II	34.21	10.92
Methanol	II	10.79	4.07
Ether	II	7.51	1.82
Acetone	II	20.87	8.20
E.G.	II	9.00	3.07
D.E.G.	II	7.18	2.67
T.E.G.	II	12.29	3.85
Cellosolve	II	5.80	2.33
Isopropyl cellosolve	II	7.90	3.55
Butyl cellosolve	II	10.38	4.36
Methyl carbitol	II	3.72	1.60
Carbitol	II	6.36	2.53
Butyl carbitol	II	11.40	3.30
P.G.	II	2.74	1.70
D.P.G.	II	6.99	2.12
H.G.	II	8.90	2.31
1-Methoxy-2-propanol	II	7.29	2.76

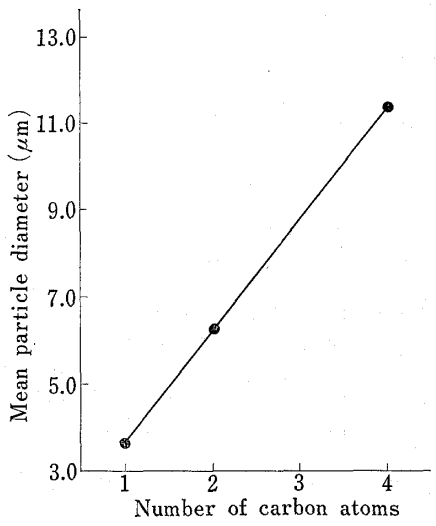

Fig. 1. Relationship between the Mean Particle Diameter and the Number of Carbon Atoms in the Hydrophobic Chain of D.E.G. and Its Derivatives used for Recrystallization

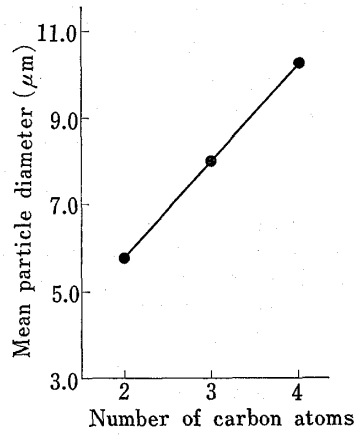

Fig. 2. Relationship between the Mean Particle Diameter and the Number of Carbon Atoms in the Hydrophobic Chain of E.G. and Its Derivatives used for Recrystallization

Figs. 1 and 2 show the volume surface mean diameters of the recrystallized particles of amobarbital plotted against the number of carbon atoms in the hydrophobic chain of the solvent for both ethylene glycol(EG) and diethylene glycol(DEG) systems. The particle diameter was 3.72 μm in the DEG system when recrystallized from methylcarbitol (with one carbon atom in the chain), 6.36 μm from carbitol, and 11.40 μm from butylcarbitol, and similar results were obtained with the EG system. Thus, the particle diameter gradually increased with increase in the number of carbon atoms, showing a linear relationship; the larger the number of carbon atoms in the hydrophobic chain, the lower the degree of pulverization.

The solubility of amobarbital in a mixture of these solvents and water 10% (w/w) at 3° is plotted against the number of carbon atoms in the hydrophobic chain of each solvent in Figs. 3 and 4. These graphs suggest that the larger the number of carbon atoms, the higher the solubility of amobarbital, resulting in the increased particle diameter shown in Figs. 1 and 2.

Thus, the results obtained from the microcrystallization of phenobarbital[10] and sulfamethizole[1] were confirmed in the EG and DEG systems, in that a higher number of carbon atoms in the hydrophobic chain reduced the degree of pulverization.

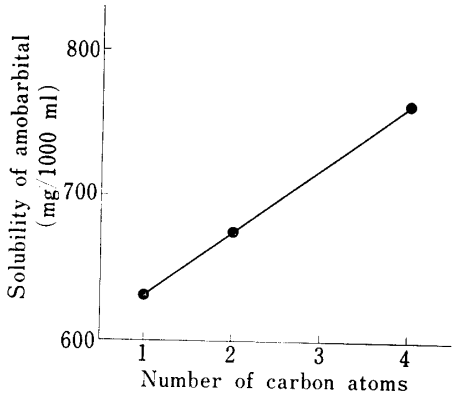

Fig. 3. Relationship between the Solubility of Amobarbital at 3° and the Number of Carbon Atoms in the Hydrophobic Chain of D.E.G. and Its Derivatives used for Recrystallization

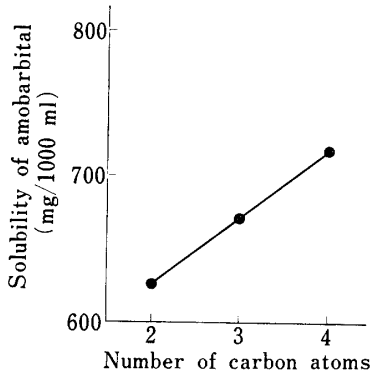

Fig. 4. Relationship between the Solubility of Amobarbital at 3° and the Number of Carbon Atoms in the Hydrophobic Chain of E.G. and Its Derivatives used for Recrystallization

The properties of microcrystals of amobarbital recrystallized from various solvents were examined. X-ray diffraction, DSC-TG, and IR data showed the presence of 2 types of crystals. Form I crystals (obtained from chloroform) were rods while form II crystals (obtained from glycols and other solvents) were plates (Fig. 5).

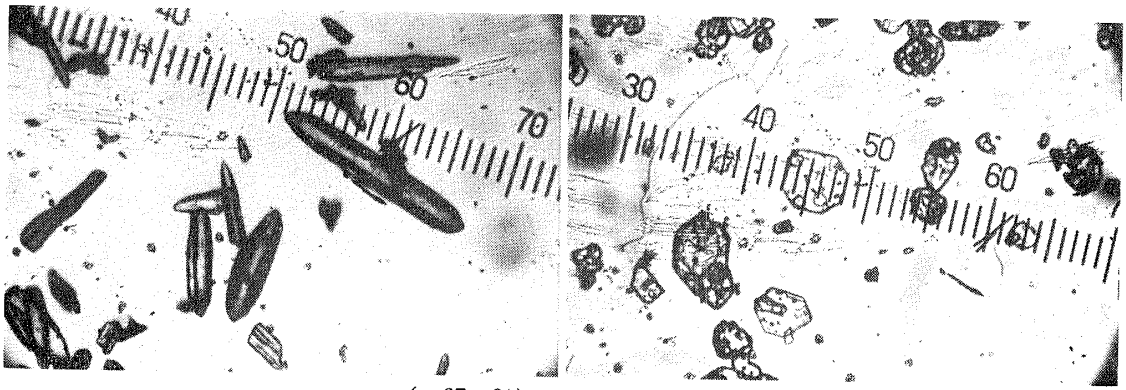

Fig. 5. Photomicrographs of Polymorphs of Amobarbital

Amobarbital (J.P.IX) was found to be form I. The X-ray diffraction pattern, IR spectrum, and DSC-TG of form I and II crystals are shown in Figs. 6, 7, and 8, respectively.

X-ray diffraction patterns were quite different in these 2 forms of the crystals, and it was presumed that there was a considerable difference in their crystal structures. The X-ray diffraction pattern of form I agreed approximately with that in the ASTM X-ray Powder Data

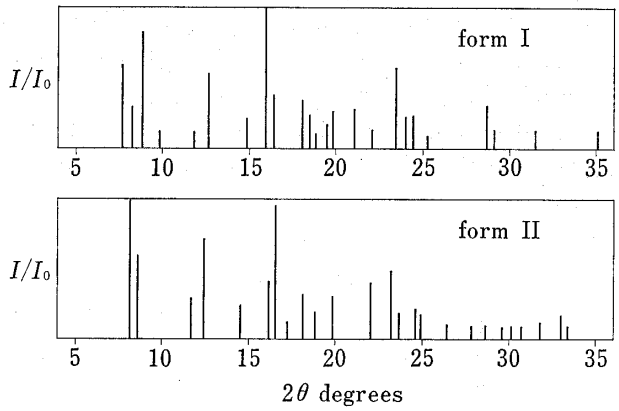

Fig. 6. X-Ray Diffraction Patterns of Polymorphs of Amobarbital

File, and that of form II agreed with the patterns reported by Williams.[11] IR spectra of these 2 kinds of crystal forms showed slight differences in the absorptions at 900—700 cm^{-1} and 1400—1300 cm^{-1}.

The results of DSC-TG also indicated differences in these 2 kinds of crystal forms. Form II recrystallized from glycols and other solvents exhibited a transition peak at 147—149° and a melting point at 156—158°, and form II changes to form I on recrystallization from chloroform. Neither a dehydration peak nor weight loss was noted for form I or form II.

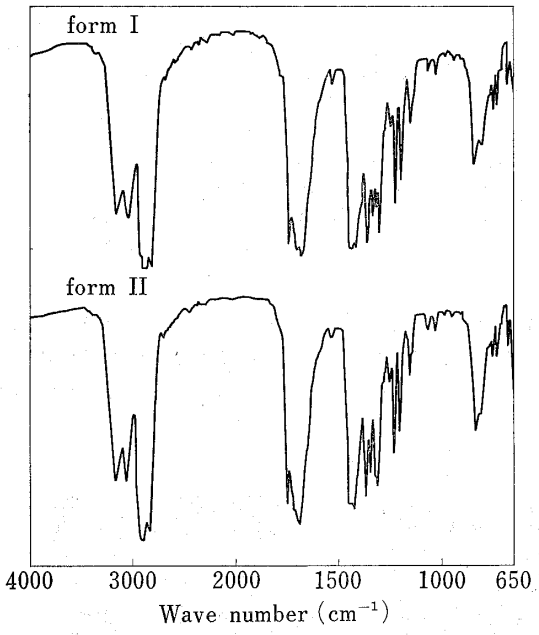

Fig. 7. IR Absorption Spectra of Polymorphs of Amobarbital in Nujol

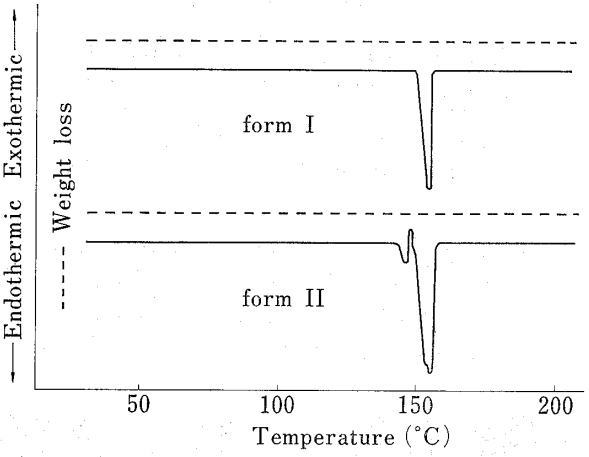

Fig. 8. DSC-TG Curves of Polymorphs of Amobarbital (heating rate: 10 K/min)
——: DSC curve, ------: TG curve.

11) P.P. Williams, *Anal. Chem.*, **31**, 140 (1959).