

5'-Methylandro-4-eno[16,17-c]isoxazolin-3-one (VIIb)—To a solution of 1.0 g. of crude VIb (m.p. 158~161°) in 50 ml. of MeOH was added 5 ml. of 10% aq. K_2CO_3 and the mixture was heated under reflux on a steam bath for 20 min. After cooling, the reaction solution was diluted with H_2O and the resulting precipitates were collected and washed with H_2O to give 0.8 g. of crude VIIb, m.p. 154~156°. Recrystallization from CH_2Cl_2 -hexane gave an analytical sample of VIIb, m.p. 159~162°, UV: λ_{max}^{EtOH} 239 m μ (ϵ 16,000). *Anal.* Calcd. for $C_{21}H_{29}O_2N$: C, 77.02; H, 8.93; N, 4.28. Found: C, 77.18; H, 8.98; N, 4.41.

The authors express their deep gratitude to Dr. S. Tatsuoka, Director of these Laboratories, for his encouragement, to Dr. Y. Abe for his guidance. Thanks are also due to Mr. H. Kamio for spectral determinations and Mr. M. Kan for elemental analysis.

Summary

5'-Methyl-5 α -andro-16,17-cis-isoxazolin-3 β -ol (Va) and 5'-methylandro-5-eno[16,17-c]isoxazolin-3 β -ol (Vb), were obtained by treatment of 3 β ,20 α -dihydroxy-5 α -pregnan-16-one 3-acetate 16-oxime (IIIa) or 3 β ,20 α -dihydroxypregn-5-en-16-one 3-acetate 16-oxime (IIIb) with tosyl chloride in pyridine followed by alkaline hydrolysis. Oxidation of Va and Vb gave the corresponding 3-oxo-compounds, VIa and VIb. VIb was further isomerized to 5'-methylandro-4-eno[16,17-c]isoxazolin-3-one (VIIb).

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166. Keiji Sekiguchi, Keiji Ito, Eiji Owada,*¹ and Keihei Ueno*²:

Studies on the Method of Size Reduction of Medicinal Compounds.

II.*³ Size Reduction of Griseofulvin by Solvation and
Desolvation Method using Chloroform (2).*⁴

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It was recently found that when griseofulvin was administered orally, its blood level was increased in proportion to the logarithm of the specific surface of the drug particles.¹⁾ Thus, the same therapeutic effect as achieved with larger crystals was demonstrated with lesser dose of finely powdered preparation of griseofulvin.²⁾ In the preceding paper, the authors reported that the particle size of the antibiotics could be reduced to a degree of several microns, if ordinary crystals of the drug were treated with chloroform or its vapor and were subsequently dried by heating in vacuum. On the basis of this simple phenomenon, a new method of size reduction of griseofulvin was successfully established on an industrial scale.

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*³ Part I: Yakuzaigaku, 23, 284 (1963).

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1) R. M. Atkinson, *et al.*: Nature, 193, 588 (1962); Antibiotics & Chemotherapy, 12, 232 (1962).

2) J. Pettit: Brit. J. Derm., 74, 62 (1962).

In the present paper, the mechanism involved in the above process was investigated, and it was confirmed that griseofulvin combines with chloroform to form a one-to-one solvated compound and the size reduction is attributed to the fact that growth of larger crystals is hindered during solvation of griseofulvin and desolvation of the solvate in solid state.

Experimental

Materials—Recrystallized griseofulvin from acetone, recrystallized antipyrine from benzene. Chloroform-treated griseofulvin was prepared by recrystallizing from chloroform or by long exposure to its vapor in a tight container.

Differential Thermal Analysis—The apparatus is the one designed and constructed by one of the authors, using a couple of matched thermistors as the detector of temperature difference.³⁾ The bath is so constructed as to permit direct observation of the sample. Recording was carried out semi-automatically, and changes of the sample during heating was noted, if they are visible.

Thermal Analysis by Visual Method—The phase diagram of the system of antipyrine and griseofulvin was constructed by the modified thaw-melt method reported previously.⁴⁾

Thermo-gravimetric Analysis—Utilizing the bath of the DTA apparatus, the weight change of the sample during heating was measured with a chemical balance at every temperature increase of 5 or 10 degrees. The sample was heated at a rate of $0.6 \pm 0.2^\circ/\text{min.}$, and reheated from one degree lower than the temperature of preceding weighing.

Dissolution in Chloroform—Five grammes of griseofulvin or the equivalent amount of the chloroform-treated product was added into an Erlenmeyer flask which was previously contained 50 ml. of distilled chloroform, and was kept at $25 \pm 0.05^\circ$ in a thermostat equipped with a shaking device. Shaking was commenced, and after certain periods, the amount of the antibiotics dissolved was determined.

Weight Increase by Exposure to Chloroform Vapor—In a tight container, maintained at $30 \pm 2^\circ$, weighing bottles each containing 100 mg. of powdered griseofulvin (60~150 mesh) and a beaker half filled with chloroform were placed side by side. At certain intervals, weight increase of each sample was measured.

Results and Discussion

The thermogram of griseofulvin has one endothermic peak due to melting, while that of the wet product obtained by recrystallization from chloroform exhibits three peaks (Fig. 1). The first one is attributable to the evaporation or boiling of free chloroform, since it disappears completely when the wet product is dried on a filter paper. The temperature of fall of the second peak is affected by heating rate and by trituration of the sample (Fig. 2). Because the fall accompanied with eutectic fusion or melting is little influenced by experimental conditions, this peak will be assigned to either the polymorphic transition of griseofulvin or the desolvation of a solvated compound. Usually, the heat of polymorphic transition is small as compared with that of melting; therefore, the second large peak should be resulted by decomposition of the solvate. As shown in Figs. 3 and 4, results of visual thermal analysis and of the differential thermal analysis (DTA) indicate that the system of antipyrine and griseofulvin belongs to the one of eutectic type, and has an eutectic point at 104° , while the mixture of antipyrine and the dry chloroform-treated product exhibits another fall at a constant temperature of $85 \sim 86^\circ$ where the liquid phase is observed to appear. It is probable that the chloroform-treated griseofulvin acts as an independent component and the liquefaction beginning at $85 \sim 86^\circ$ will be attributed to eutectic fusion of the system of antipyrine and the chloroform-treated griseofulvin.

The combining ratio of chloroform and the antibiotics in the solvate can be determined by the thermo-gravimetric analysis (TGA) (Fig. 5). The fact that the weight of

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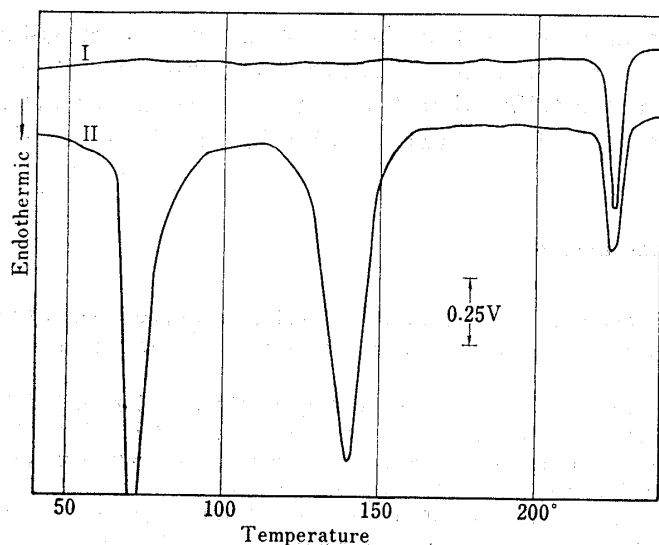


Fig. 1. DTA Curves of Griseofulvin and Griseofulvin Chloroformate

Sample: I, powdered Gr., 66 mg.
 II, wet CHCl_3 -treated product
 Sensitivity: 2.5 V Heating rate: $4^\circ/\text{min.}$
 Reference: freeze-dried KCl, 75 mg.

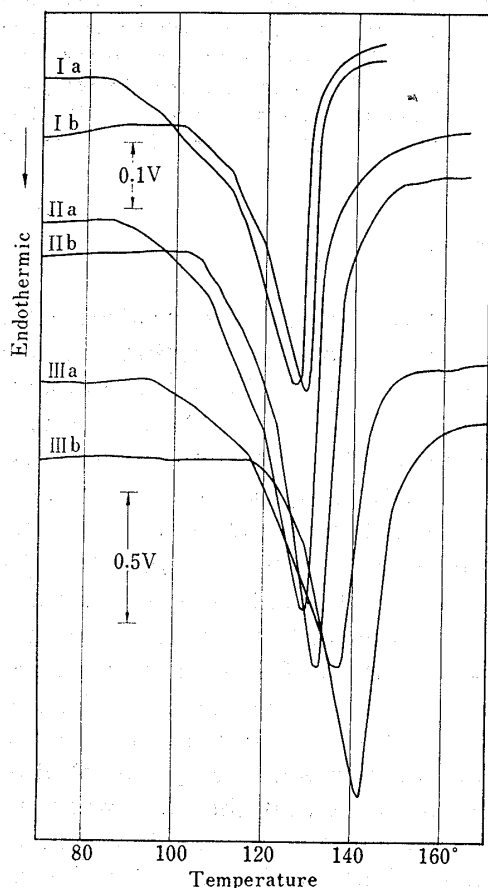


Fig. 2. Influence of Experimental Conditions on Desolvation of Griseofulvin Chloroformate

Experimental Conditions	Ia	IIa	IIIa	Ib	IIb	IIIb
Sample state	powdered			not powdered		
Sample amount (mg.)	103	99	114	102	98	110
Sensitivity (V)	1	1	2.5	1	1	2.5
Heating rate ($^\circ/\text{min.}$)	1	2	5	1	2	5

Reference: freeze-dried KCl, 100 mg.

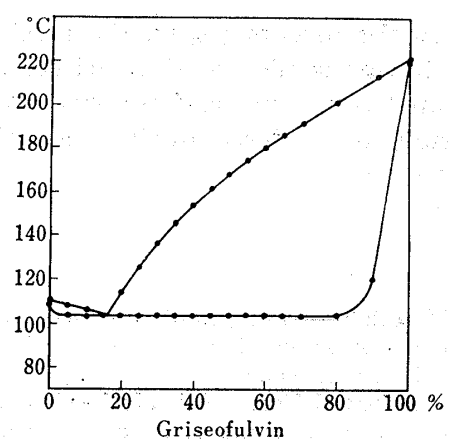


Fig. 3. Phase Diagram of Antipyrine and Griseofulvin System

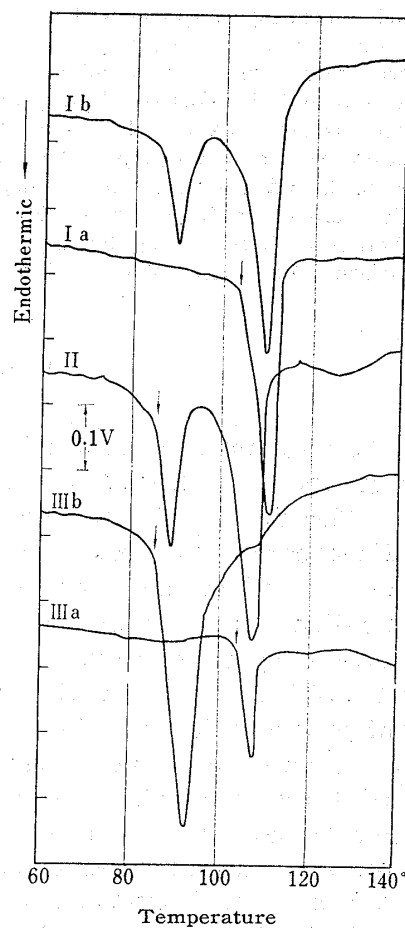


Fig. 4. DTA Curves of Antipyrine-Griseofulvin and Antipyrine-Griseofulvin Chloroformate Mixtures

Experimental Conditions	Antipyrine-Gr. Mixt.		Antipyrine-Gr. $\cdot\text{CHCl}_3$ Mixt.		
	Ia	IIIa	Ib	II	IIIb
A/G (mol. ratio)	0.13	1.00	0.13	0.20	1.00
Sample amount (mg.)	71	63	65	100	52
Heating rate ($^\circ/\text{min.}$)	2	1	2	1	2

Sensitivity: all 1 V
 Reference: freeze-dried KCl, 75 or 100 mg.
 The arrows show the points where the cell wall becomes cloudy.

griseofulvin itself is observed to be constant from 50° to 240° indicates that the substance shows negligible vapor pressure even in liquid state. Small change in weight at the initial period of heating will be due to the departure of adhering moisture. On the other hand, the behavior of wet chloroformate during heating is entirely different from the antibiotics itself and the TGA curve corresponds to the result of DTA. Due to evaporation of free chloroform, weight decrease is observed at the beginning. Then, the weight of the sample remains constant until the temperature is raised to about 95°. By further rise of temperature, abrupt change in weight begins to occur, showing decomposition of the solvate. When the temperature reaches at about 130°, the weight becomes constant again. Since the difference between the two horizontal lines is the amount of chloroform involved in the solvate, the combining ratio can be calculated precisely as one to one. The same ratio was also obtained with elemental analysis of the dry solvate. *Anal.* Calcd. for $C_{17}H_{17}O_6Cl \cdot CHCl_3$: C, 45.79; H, 3.84. Found; C, 45.82; H, 3.85.

From these experimental evidences, it becomes clear that formation and decomposition of the chloroformate play an important role in reducing the size of griseofulvin. When the drug is treated with chloroform, disintegration of the rigid crystals occurs in a little while as a result of conversion of crystal structure. Because the antibiotics itself is less stable in chloroform solution, the dissolution curve once shows a peak before the concentration attains a constant value of the solubility of the chloroformate (Fig. 6). Thus, the first step of size reduction by liquid chloroform will be less effective than by vapor, since in the

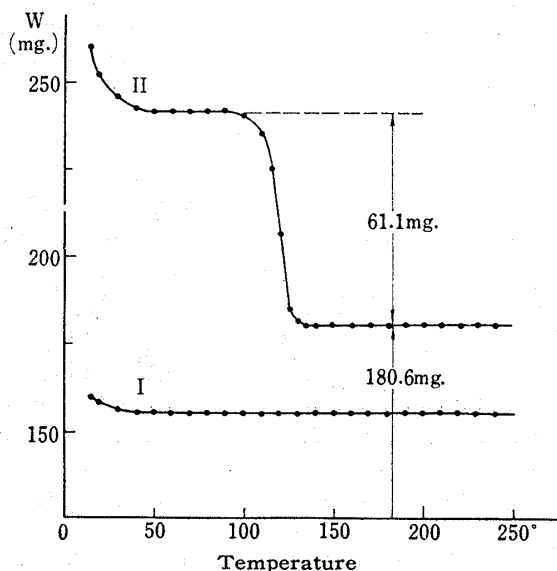


Fig. 5. TGA Curves of Griseofulvin and Griseofulvin Chloroformate

Sample: I, griseofulvin; II, wet chloroformate
Heating rate: $0.6 \pm 0.2^\circ/\text{min.}$
The combining ratio of Gr. and $CHCl_3$ is calculated as $5.119 \times 10^{-4} : 5.118 \times 10^{-4} = 1 : 1$.

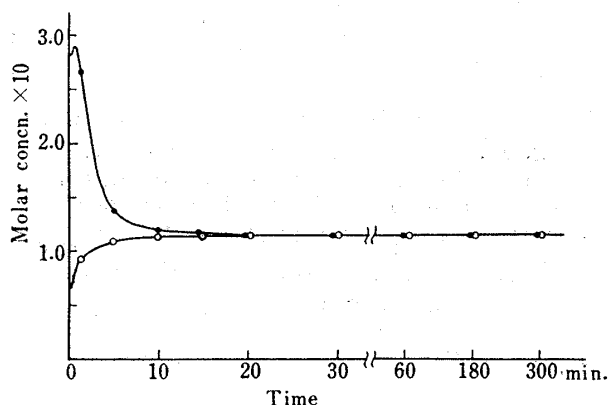


Fig. 6. Dissolution Curves of Griseofulvin and Griseofulvin Chloroformate in Chloroform at $25 \pm 0.05^\circ$

Closed circles: griseofulvin (60~150 mesh)
Open circles: griseofulvin chloroformate (60~150 mesh)
Shaking: twice per second with an amplitude of 18 mm.
Solubility at 25° : $1.15 \pm 0.01 \times 10^{-3} \text{ mol./L.}$

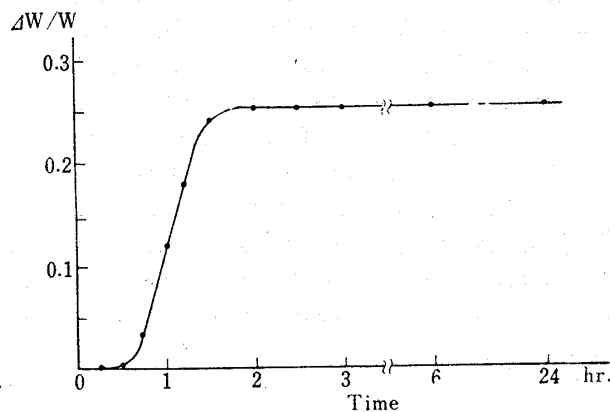


Fig. 7. Weight Increase of Griseofulvin by Exposure to Chloroform Vapor

Sample: 100 mg. each, 60~150 mesh;
Temperature: $30 \pm 2^\circ$
At equilibrium, the stoichiometric ratio of $CHCl_3$ to griseofulvin is calculated as 0.992.

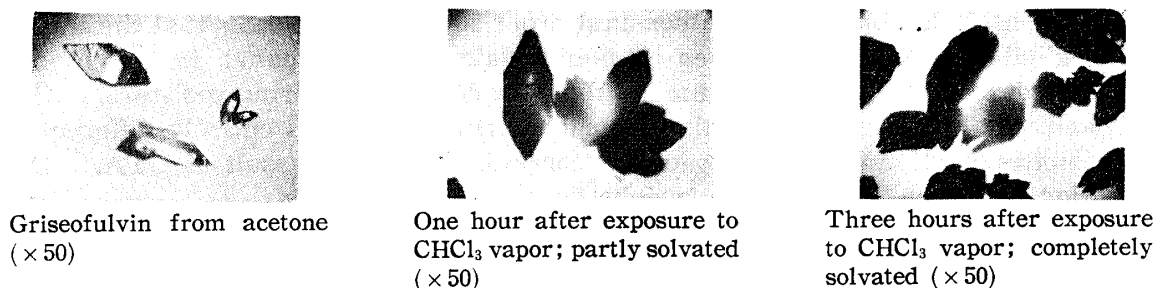


Fig. 8. Features of Size Reduction during Solvation

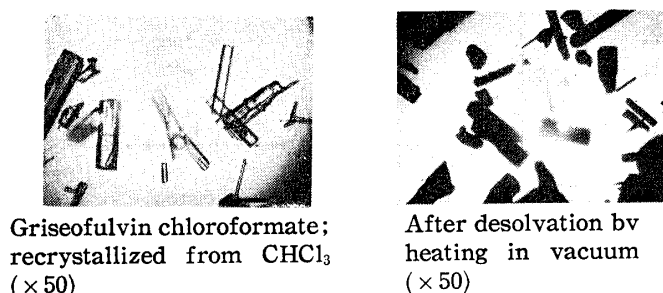
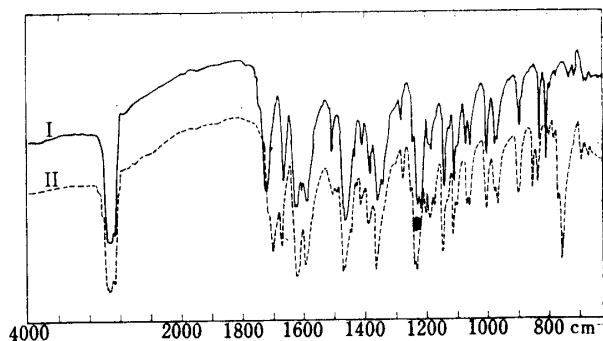


Fig. 9. Features of Size Reduction during Desolvation

process proceeds rather rapidly (Fig. 7). The rate of the second step, desolvation, becomes rapid by heating in vacuum. Size reduction is also accomplished at this stage, since in solid state, growth of new crystals is largely hindered by the difficulty of migration of the desolvated molecules to reach the crystal nuclei. In this connection, it is supposed that the low vapor pressure of griseofulvin will be related to the growth of minute crystals. In Figs. 8 and 9, features of size reduction by solvation and desolvation are illustrated.

Difference in infrared absorption spectra of griseofulvin and the chloroformate measured as a solid ground in nujol is observed in several regions (Fig. 10). The latter shows a strong band at 750 cm^{-1} , which is indicative of the presence of chloroform moiety. The band of griseofulvin at 1720 cm^{-1} which is attributable to the CO group in the benzofuran ring shows a fall of 20 cm^{-1} by solvation. At the same time, solvation results in a rise in the CO frequency of cyclohexene ring by 5 cm^{-1} with no appreciable shift of the C=C band. Although conclusive evidence for the nature of the bonding is not available, the dipole-induced dipole interaction between griseofulvin and chloroform is supposed to take part in the bond.

Fig. 10. Infrared Spectra of Griseofulvin and Griseofulvin Chloroformate (in Nujol)
I : Griseofulvin ; II : Griseofulvin chloroformate

Conclusion

It is generally accepted that particle size and particle size distribution are important factors in the absorption of a drug, since dissolution rate is directly related to the surface area available for exposure to the solvent. Clinical efficacy of an insoluble drug will be improved by administering a finely powdered preparation. For this reason, size

reduction often becomes a matter of importance for the pharmaceutical industry. For such a rigid material as griseofulvin, uniform subdivision to micron grade will not be attained by mechanical processes, such as grinding and crushing. Although the method of precipitation by jetting a solution into an insoluble solvent is adopted for preparing fine powder of the antibiotics,⁵⁾ the cost increase of the product will not be neglected. The method first proposed in this series of papers is not only simple but also economical, and is applied successfully for the production of finely divided griseofulvin. Increase in cost by insertion of this process is negligible. Also, the product shows therapeutical excellence to the ordinary one.

The authors suggested previously that particle size can be largely reduced by freeze-drying of a solution containing a drug substance less than the eutectic composition of the system between the drug and a suitable solvent.⁶⁾ So far as the authors know, the "freeze-drying" and the "solvation and desolvation" method are entirely different from those hitherto adopted in pharmaceutical engineering. Although these methods are selective and are restricted by the chemical properties of the materials to be pulverized, the practical success in the production of finely powdered griseofulvin will give promise of further applications, especially to organic medicinals.

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Summary

The mechanism of the size reduction of griseofulvin to micron grade caused by exposing to liquid or vaporous chloroform and then removing it in a vacuum at higher temperatures was investigated by the differential thermal and thermo-gravimetric analysis. The results indicate that a solvated compound having a molar composition of 1:1 is formed between the antibiotics and chloroform. It becomes clear that the formation and decomposition of the solvate play an important role in reducing particle size, since lattice rearrangement to the solvate and back to the original compound is occurred during these processes, and the growth of larger crystals is prevented by the difficulty of migration of the newly formed molecules to the nuclei in the solid state. The "solvation and desolvation" method of size reduction first acquired practical success in the production of finely powdered griseofulvin is thought to find application to other materials, since the processes involved are simple, effective and economical.

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6) K. Sekiguchi, N. Obi, Y. Ueda : This Bulletin, 12, 134 (1963).