Effect of Dose, pH, and Osmolarity on Nasal Absorption of Secretin in Rats. III.[1,2] *In Vitro* Membrane Permeation Test and Determination of Apparent Partition Coefficient of Secretin

Takayuki OHWAKI,* Masaaki ISHII, Shigeru AOKI, Kimio TATSUISHI, and Masanori KAYANO

Research Laboratories of Pharmaceutical Development, Eisai Co., Ltd., Takehaya-machi, Kawashima-cho, Hashima-gun, Gifu 483, Japan.
Received March 11, 1989

Nasal absorption of secretin in rats was enhanced in an acid solution and the maximum absorption was observed at a sodium chloride solution molarity of 0.462. In order to predict how changes in the secretin molecule would affect its absorption through the nasal mucosa independently of structural changes in the epithelial membrane, an artificial membrane permeation test was conducted, and the apparent partition coefficient between octanol and a test solution was determined. The concentration of secretin was measured using high performance liquid chromatography. The amount of secretin that permeated through an artificial membrane was hardly affected by changes in pH, which suggested that the size of the secretin molecule was not changed. The apparent partition coefficient, however, increased as the pH of the test solution rose from 3.81 to 7.0, which suggested that the hydrophobicity of secretin was enhanced. In relation to the osmolarity of the test solution, the amount of permeation was hardly affected by the concentration of sodium chloride, but the partition coefficient increased with the concentration of the sodium chloride solution. It was supposed that the size of the secretin molecule was not changed in spite of the increasing hydrophobicity, and the nasal absorption of secretin at a sodium chloride molarity of 0.462 was dependent on a change in the epithelial cells. When sorbitol was used as an osmoregulatory agent, the apparent partition coefficient hardly varied as the osmolarity of the solution was increased, whereas the amount of permeation decreased, and these findings reflected the nasal absorption in rats.

Keywords secretin; membrane permeation test; apparent partition coefficient; nasal absorption; rat

In our previous studies,[1,2] in order to ascertain the feasibility of a nasal dosage form of secretin, we investigated the intranasal administration to rats of secretin, a hormone secreted in the digestive tract and used clinically for the treatment of duodenal ulcer.[3-10]

It was noted that the absorbability was one-tenth of the bioavailability after intravenous administration, and was affected by the pH and osmolarity of the secretin preparations. It can be assumed that the effect of pH is mainly due to structural changes in the epithelial membrane. However, the results of the osmolarity effect study revealed that the absorption decreased in proportion to the increase in the molar concentration of the sorbitol solution, and the maximum absorption was observed at a sodium chloride solution molarity of 0.462, at which shrinkage of epithelial cells was observed.

It has been reported that a highly water-soluble drug can be absorbed by the paracellular route through the mucosa,[11,12] and so secretin, being highly water-soluble is assumed to be absorbed mainly through the paracellular route.

It is well known that the molecular volume is one of the principal factors controlling the absorption of drugs through the paracellular route,[13] and that lower bioavailability results from a larger molecular volume of a drug. The molecular volume of a drug can chang; for example, the polypeptide drug insulin is reported to self-associate depending upon insulin concentration, pH, solvent composition, ionic strength, and solvent dielectric properties.[14]

The objective of this study was to predict, by means of artificial membrane permeation tests and determination of the apparent partition coefficient, how changes in the secretin molecule would affect its absorption through the nasal mucosa, independently of structural changes in the epithelial membrane.

Experimental

Materials A 24000 CHR unit/mg (Crick, Happer and Raper unit/mg) preparation of synthetic secretin (Eisai Co., Ltd., Tokyo) was used in this study. The other chemicals employed were of analytical or reagent grade.

Polycarbonate membrane (Nomura Micro Science K.K., Tokyo) was used as a test membrane. It was confirmed under a scanning electron microscope that the pore size of the membrane was not changed by either the pH or osmolarity of the test solutions during a permeation test.

Apparatus for Drug Permeation The permeability cell apparatus shown in Fig. 1 was used in this permeation test.

The two ports of the donor and receptor cells were held together by a clamp. The volume of both cells was 20 ml, and both have a sampling opening of 1.35 cm in diameter and a cell opening providing an effective diffusional area of 0.724 cm^2. A test membrane was inserted and secured between the membrane holders of the cell orifices. In order to prevent contamination of the test solution with the heating water in the bath, silicon grease was spread over the surfaces of the membrane holder of both cells before the test membrane was inserted.

Two magnetic stirrers were used to agitate the test solution in the cells, and the permeation cell apparatus was immersed in a water bath maintained at 37 °C.

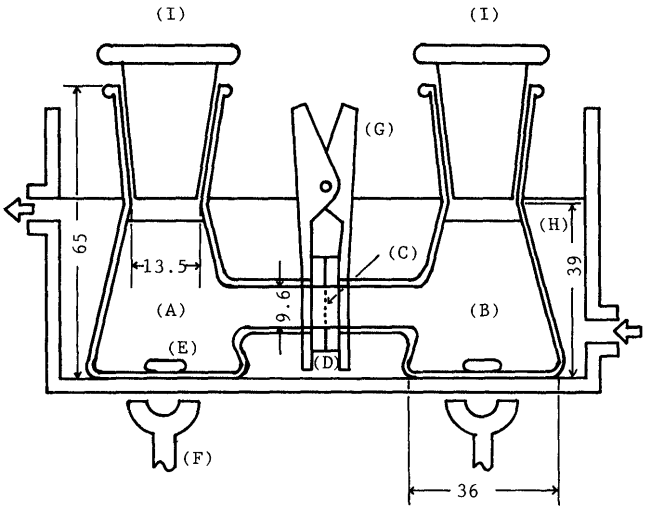

Fig. 1. Apparatus for Drug Permeation Test

(A) Donor cell; (B) receptor cell; (C) membrane; (D) silicon grease; (E) stirrer bar; (F) magnetic stirrer; (G) clamp; (H) water bath; (I) sampling opening (dimensions are in mm).

Preparation of Test Solutions For the pH dependency experiments, solutions of isotonic 0.236 M citric acid: 0.123 M disodium phosphate buffer (Wako Pure Chemicals Industries, Ltd., Osaka) ranging in pH from 2.1 to 6.33, and isotonic 0.171 M potassium phosphate: 0.144 M sodium acid carbonate buffer (Wako) solutions of pH values between 7 to 8 were prepared.

For the osmolarity dependency experiments, sodium chloride was dissolved to give molar concentrations from 0 to 1.078, and sorbitol was dissolved to yield molar concentrations between 0 and 2.156.

In order to prevent the adsorption of secretin on the walls of utensils, 0.05% (w/v) of bovine serum albumin (Seikagaku Kogyo Co., Ltd., Tokyo) was added to the test solution.

The pH of each solution was measured with a pH meter (model HM-5B, Toa Electronics Ltd., Tokyo), and the osmolarity with an osmometer (model 3W, Advanced Instrument Inc., MA).

Permeation Test About 3 mg of secretin was weighed precisely and was put into the donor cell prior to the start of the test. Then 15 ml of test solution, heated in advance to 37 °C, was poured into both cells (Fig. 1). The test was started by stirring at a constant rate. Aliquots of 10 μl of test solution were withdrawn periodically from the donor cell and aliquots of 50 μl from the receptor cell, and the secretin concentration was measured by high-performance liquid chromatography (HPLC).

In these studies, conducted at least in duplicate, the range of measured values was found to be less than 0.3% of the total amount.

Selection of Membrane Pore Size Experiments were conducted according to the permeation test procedure described above with membrane pore sizes of 0.05, 0.1 and 0.4 μm in order to select a membrane pore size suitable for the permeation test.

Sodium chloride solutions with concentrations of 0.154 and 0.462 M and sorbitol solutions with concentrations of 0.308 and 0.924 M, prepared as described above, were employed in this experiment.

As Fig. 2 indicates, a pore size of 0.05 μm was too small for differences in secretin concentration to be detected in the receptor cell under the various test conditions, and a similar lack of detection sensitivity was seen with a pore size of 0.4 μm, which was too large. Accordingly, a pore size of 0.1 μm was chosen for this permeation test.

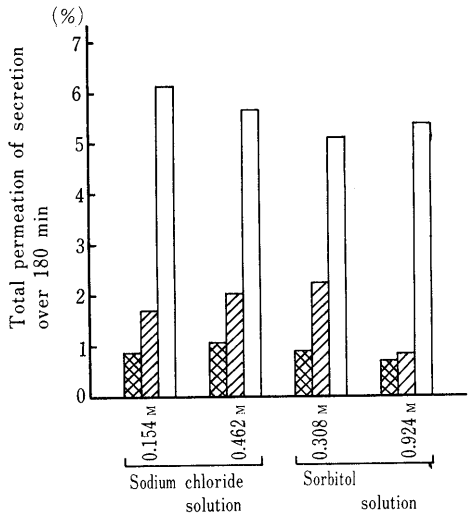

Fig. 2. Total Permeation of Secretin over 180 min at Various Membrane Pore Sizes

The data are expressed as means ($n=2$). ▨, 0.05 μm; ▨, 0.1 μm; □, 0.4 μm.

TABLE I. HPLC System and Analytical Conditions

Apparatus	
Pump	LC-3A reciprocating pump (Shimazu)
Detector	SPD-2A spectrophotometer (Shimazu)
Injector	Rheodyne 7125 injector
Conditions	
Column	Unisil C_{18} 3μ, i.d. 4.6 × 50 mm (Gasukuro Kogyo Inc.)
Mobile phase	CHCN : H_2O : 70% $HClO_4$ = 37 : 63 : 0.5
Wavelength	210 nm
Temperature	30 °C
Flow rate	1 ml/min
Injection volume	10, 50 μl

Determination of Apparent Partition Coefficients The apparent partition coefficients of secretin between octanol and the test solutions were determined by the following procedure.

About 0.25 mg of secretin was weighed precisely and placed in a glass centrifuge tube. The test solution (5 ml) was poured into the tube and the secretin was dissolved. After the addition of 5 ml of octanol, the solution was shaken vigorously for 1 h and then centrifuged for 20 min at 3000 rpm. The tube was left to stand for 24 h, and a sample for analysis was afterwards withdrawn from the water layer. The whole procedure was carried out in triplicate at 25 °C.

Assay The analytical procedure used to determine the secretin concentration was a modification of the method described by Asakawa *et al.*[15,16]

For the permeation test, 10 μl of the test solution was sampled from the donor cell, and 50 μl from the receptor cell. The secretin concentration of each sample was measured by HPLC without further treatment of the sample. The HPLC system and analytical conditions are shown in Table I. A linear relationship between the peak height and the secretin concentration was obtained in the concentration range of 9.9×10^{-4} to 1.98×10^{-2} mg/ml under the conditions shown in Table I.

For the determination of the apparent partition coefficient, the same HPLC system and analytical conditions were employed to measure the secretin concentration in the test solution layer.

It was confirmed in advance by HPLC under the conditions shown in Table I that secretin was stable in each test solution during each experiment.

Results and Discussion

The membrane permeation test and the determination of the apparent partition coefficients of secretin were conducted to predict how changes of the secretin molecule (independently of the structural changes of the epithelial membrane) would affect its absorption through the nasal mucosa.

Figure 3 shows the time course of the permeation of secretin from the donor to the receptor cell at various pH values. The amount that permeated increased linearly with time, and was largest at pH 8.

It is well known that the permeation of a drug can be simulated by a pseudo-first-order equation. However, in the present study, the amount permeating increased linearly

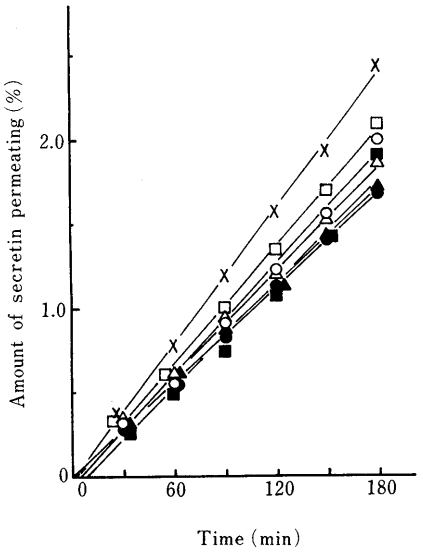

Fig. 3. Amount of Permeation of Secretin from the Donor Cell to the Receptor Cell at Various pH Values

○, pH 2.1; ●, pH 2.94; △, pH 3.81; ▲, pH 4.79; □, pH 6.33; ■, pH 7.00; ×, pH 8.00. The data are expressed as means ($n=2$).

TABLE II. Linear Regression Parameters[a] for the Results of Membrane Permeation Tests of Secretin at Various pH Values

pH	$A^{b)}$	$B^{c)}$	$r^{d)}$
2.10	0.010911	−0.047536	0.9973
2.94	0.009314	−0.004143	0.9999
3.81	0.010182	−0.005179	0.9998
4.79	0.009375	0.016821	0.9997
6.33	0.011577	−0.025678	0.9987
7.00	0.010255	−0.083607	0.9942
8.00	0.013345	−0.016500	0.9994

a) Linear regression was conducted using the following equation, Amount of permeated secretin (%) = $A \times$ time (min) + B. *b*) Slope (apparent permeation rate in %/min). *c*) Intercept at time = 0. *d*) Correlation coefficient.

TABLE III. Apparent Partition Coefficients of Secretin between Octanol and Buffer Solutions

pH	C_{oct}[a)] (mg/ml)	C_b[b)] (mg/ml)	$C_{oct}/C_b(-)$
2.10	0	0.0570	0
2.94	0	0.0612	0
3.81	0.00127	0.0392	0.0324
4.79	0.00203	0.0510	0.0398
6.33	0.00240	0.0501	0.0479
7.00	0.00740	0.0391	0.1893
8.00	0.00500	0.0340	0.1282

a) Secretin concentration in the octanol layer was calculated by subtracting the concentration in the buffer solution layer from the initial concentration in that layer. *b*) Secretin concentration in buffer solution layer. The data are expressed as means ($n=3$).

with time, as shown in Fig. 3. Linear regression was conducted to determine the apparent permeation rate. Table II shows the linear regression parameters for the results of the membrane permeation tests of secretin at various pH levels. The permeation rate at pH 8 was the largest, but those at the other pH values were almost the same.

In order to assess the hydrophobicity of secretin at different values of pH, the apparent partition coefficients were measured. Table III shows those of secretin between octanol and buffer solutions of various pH. The secretin concentration in the octanol layer at pH 2.10 and 2.94 was zero and the apparent partition coefficient rose with pH from 3.81 to 7.0. It was suggested that the hydrophobicity of secretin could be altered by changes in the pH.

In our previous studies,[1,2] the absorption on intranasal administration of the active preparation of secretin dissolved in a buffer solution was enhanced in an acid solution and was larger than that for the pretreatment effect below a pH of 4.79. It was smaller than that for the pretreatment effect at pH values between 7 to 8, which suggested that the effect of pH on the nasal absorption of secretin was due mainly to structural changes in the epithelial membrane, but also to changes in the secretin molecule or in the electric charge on the epithelial surface. Since the results of these experiments show that the artificial membrane permeation of secretin was hardly altered by changes in pH and that the hydrophobicity of secretin rose slightly as the pH increased, the nasal absorption of secretin was thought to be regulated mainly by structural changes in the epithelial membrane, so that the results of the pretreatment study were probably not caused by changes in the size of the secretin molecule.

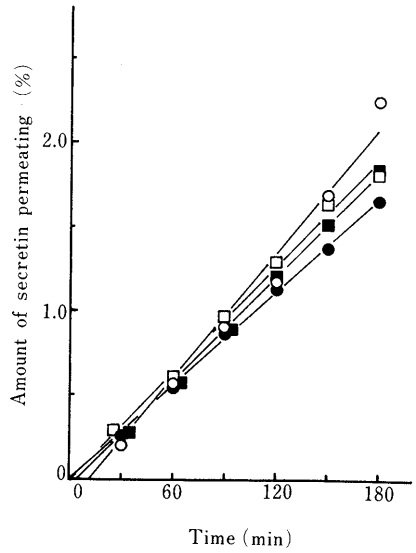

Fig. 4. Amount of Permeation of Secretin from the Donor Cell to the Receptor Cell at Various Concentrations of Sodium Chloride

○, 0 M; ●, 0.154 M; □, 0.462 M; ■, 1.078 M. The data are expressed as means ($n=2$).

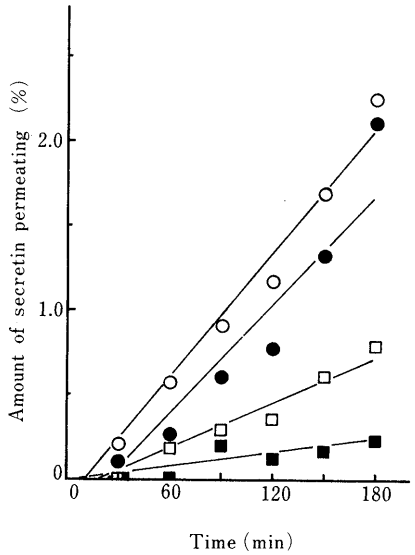

Fig. 5. Amount of Permeation of Secretin from the Donor Cell to the Receptor Cell at Various Concentrations of Sorbitol

○, 0 M; ●, 0.308 M; □, 0.924 M; ■, 2.156 M. The data are expressed as means ($n=2$).

On the other hand, to elucidate how the artificial membrane permeation and the hydrophobicity of the secretin molecule would be altered by variations in osmolarity, the permeation test and measurement of the apparent partition coefficient were carried out using sodium chloride or sorbitol as an osmoregulatory agent.

Figures 4 and 5 show the time course of the permeation of secretin from the donor cell to the receptor cell at various osmolarities of solutions prepared with sodium chloride or sorbitol as the osmoregulatory agent, respectively.

In the case of sodium chloride, as shown in Fig. 4, the amount of secretin that permeated was hardly altered by the osmolarity, and the apparent permeation rate was not altered (Table IV).

However, the amount of permeation decreased as the

Table IV. Linear Regression Parameters[a] for Results of Membrane Permeation Tests of Secretin at Various Concentrations of NaCl

NaCl (M)	$A^{b)}$	$B^{c)}$	$r^{d)}$
0	0.012275	−0.134464	0.9893
0.154	0.009231	0.002643	0.9995
0.462	0.010526	−0.003786	0.9978
1.078	0.010277	−0.023268	0.9998

a) Linear regression was conducted according to the following equation. Amount of permeated secretin (%) = $A \times$ time (min) + B. *b*) Slope (apparent permeation rate in %/min). *c*) Intercept at time = 0. *d*) Correlation coefficient.

Table V. Linear Regression Parameters[a] for Results of Membrane Permeation Tests of Secretin at Various Concentrations of Sorbitol

Sorbitol (M)	$A^{b)}$	$B^{c)}$	$r^{d)}$
0	0.012275	−0.134464	0.9893
0.308	0.011137	−0.259179	0.9477
0.924	0.004486	−0.086000	0.9730
2.156	0.001339	−0.009964	0.8816

a) Linear regression was conducted according to the following equation. Amount of permeated secretin (%) = $A \times$ time (min) + B. *b*) Slope (apparent permeation rate in %/min). *c*) Intercept at time = 0. *d*) Correlation coefficient.

sorbitol concentration rose, as shown in Fig. 5, and the apparent permeation rate fell at the same time (Table V). This suggested that the size of the secretin molecule changes with the concentration of sorbitol solution, but this is not the case with sodium chloride solution.

Tables VI and VII show the apparent partition coefficients of secretin between octanol and various concentrations of sodium chloride or sorbitol solution. As Table VI indicates, the apparent partition coefficients increased with the molarity of sodium chloride in the range of 0.154 to 1.078 M, but rose only slightly at a sorbitol molarity of 2.156 (Table VII).

In the previous studies,[1,2] the maximum nasal absorption of secretin in rats was observed at a sodium chloride solution molarity of 0.462 and a similar profile was obtained in the pretreatment study, while it was decreased in sorbitol solution as the osmolarity increased. From the results of histological examination of the nasal mucosa in rats,[2] shrinkage of epithelial cells occurs at a sodium chloride solution concentration of 0.462 M, while no structural change was observed with a 0.924 M sorbitol solution.

The results of this study suggested that the size of the secretin molecule was not changed, but the hydrophobicity of secretin was altered as the sodium chloride solution concentration was changed. However, the results of the present and previous studies[1,2] indicate that the nasal absorption of secretin at a sodium chloride molarity of 0.462 was dependent on a change (shrinkage) in the epithelial cells, irrespective of the secretin hydrophobicity and the osmolarity of sodium chloride solution. It was considered that the alteration of the apparent partition coefficients of secretin between octanol and sodium chloride solutions in the range of 0.0357 to 0.9584 did not affect the nasal absorption of secretin, and also that the efflux through the nasal mucosa was not influenced by the change of sodium chloride solution osmolarity.

Table VI. Apparent Partition Coefficients of Secretin between Octanol and Sodium Chloride Solutions

NaCl (M)	pH	Osmo[a] (mOsm)	$C_{oct}{}^{b)}$ (mg/ml)	$C_{sc}{}^{c)}$ (mg/ml)	C_{oct}/C_{sc} (−)
0	5.26	0	0.00160	0.03740	0.0428
0.154	5.09	277	0.00240	0.06710	0.0357
0.462	5.02	822	0.01520	0.05280	0.2879
1.078	4.90	1948	0.02765	0.02885	0.9584

a) Osmolarity. *b*) Secretin concentration in the octanol layer was calculated by subtracting the concentration in the sodium chloride solution layer from the initial concentration in that layer. *c*) Secretin concentration in sodium chloride solution layer. The data are expressed as means ($n = 3$).

Table VII. Apparent Partition Coefficients of Secretin between Octanol and Sorbitol Solution

Sorbitol (M)	pH	Osmo[a] (mOsm)	$C_{oct}{}^{b)}$ (mg/ml)	$C_{s}{}^{c)}$ (mg/ml)	C_{oct}/C_{s} (−)
0	5.26	0	0.00160	0.03740	0.0428
0.308	5.43	306	0	0.05820	0
0.924	5.37	921	0	0.06500	0
2.156	5.74	1940	0.00120	0.05630	0.0213

a) Osmolarity. *b*) Secretin concentration in the octanol layer was calculated by subtracting the concentration in the sorbitol solution layer from the initial concentration in that layer. *c*) Secretin concentration in sorbitol solution layer. The data are expressed as means ($n = 3$).

In the case of sorbitol solutions, the diminution of the nasal absorption of secretin in rats appeared to result from the decreased permeation through the nasal mucosa.

Acknowledgement The authors are grateful to Mrs. Sachiko Takahashi at the Research Laboratories of Pharmaceutical Development, Eisai Co., Ltd., for measuring the osmolarity of the test solutions.

References and Notes

1) T. Ohwaki, H. Ando, S. Watanabe, and Y. Miyake, *J. Pharm. Sci.*, **74**, 550 (1985).
2) T. Ohwaki, H. Ando, F. Kakimoto, K. Uesugi, S. Watanabe, Y. Miyake, and M. Kayano, *J. Pharm. Sci.*, **76**, 695 (1987).
3) H. Matsuo, A. Seki, and T. Ishikawa, *Horumon To Rinsho*, **23**, 507 (1975).
4) M. I. Grossman, *Gastroenterology*, **50**, 912 (1966).
6) A. Miyoshi, K. Fujii, T. Okuhara, T. Suyama, T. Kai, T. Kawagoe, and T. Inagawa, *Shinryo To Shinyaku*, **11**, 1833 (1974).
7) S. J. Konturek, *Gut*, **14**, 842 (1973).
8) S. J. Konturek, T. Radecki, P. Thor, and N. Kwiecien, *Am. J. Dig. Dis.*, **18**, 135 (1973).
9) K. Kawai, F. Misaki, T. Miyaoka, K. Kimoto, K. Shimamoto, M. Takebayashi, M. Nakajima, Y. Mitsuyoshi, and K. Fukumoto, *Gendai No Rinsho*, **8**, 177 (1974).
10) K. Nakamura, *Shinryo To Shinyaku*, **11**, 1929 (1974).
11) A. Karino, M. Hayashi, S. Awazu, and M. Hanano, *J. Pharmacobio-Dyn.*, **5**, 670 (1982).
12) Y. Kaneo, *Acta Pharm. Suec.*, **20**, 379 (1983).
13) A. N. Fisher, K. Brown, S. S. Davis, G. D. Parr, and D. A. Smith, *J. Pharm. Pharmacol.*, **39**, 357 (1987).
14) S. Sato, C. D. Ebert, and S. W. Kim, *J. Pharm. Sci.*, **72**, 228 (1983).
15) N. Asakawa, M. Tsuno, Y. Saeki, M. Matsuda, T. Hattori, M. Ueyama, A. Shinoda, and Y. Miyake, *Yakugaku Zasshi*, **102**, 43 (1982).
16) N. Asakawa, M. Tuno, Y. Saeki, F. Kakimoto, T. Hattori, M. Ueyama, A. Shinoda, and Y. Miyake, *Yakugaku Zasshi*, **103**, 518 (1983).