[Chem. Pharm. Bull. 23(12)3088—3094(1975)]

UDC 547.581'26.03 : 547.37.03

Interaction of Methyl *p*-Hydroxybenzoate with Polyoxyethylene Dodecyl Ethers[1)]

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(Received January 25, 1975)

The interaction of methyl *p*-hydroxybenzoate (MP) with polyoxyethylene dodecyl ethers having a variety of numbers of oxyethylene units in the homogeneous chain was investigated by ultrafiltration and nuclear magnetic resonance spectrometry. The results obtained suggested the existence of two kinds of interaction mechanisms between the preservative and the nonionic surfactant micelle. The binding parameters in each class were calculated using a multiple regression technique. In the primary class of sites, the interaction obeyed the form of the Langmuir type adsorption and the bound molecules of MP were probably located at the interface of the hydrocarbon core and the polyoxyethylene mantle of the micelles. Moreover, in the second class of sites, the interaction could be described as a simple partition between the micelles and the aqueous phase and the preservative molecules seemed to be situated in the polyoxyethylene mantle.

The interaction between the preservatives and the micelles of nonionic surfactants of the polyoxyethylene type has been the subject of many investigations. One main interest has been the inactivation of preservatives in the presence of nonionic surfactants. It is generally accepted that the preservative molecules solubilized or bound within the surfactant micelles are inactive and, although the micelles act as a reservoir of the preservative, the antimicrobiological activity is governed by the concentration of the unbound or free preservative in the intermicellar aqueous phase.[3)] On the mechanism of these interactions, the literature is usually made to either micellar solubilization of the phenolic preservative or to the formation of hydrogen bonded complexes between polyoxyethylene chains and phenolic compounds. Kostenbauder[4)] maintains that it is unnecessary to distinguish between these mechanisms since micellar solubilization can be considered to fall within the broad scope of the complex formation described by Higuchi and co-workers.[5)]

Many methods available for the quantitative investigation of the interaction between the preservatives and the nonionic surfactant micelles include the solubility method,[3b,6)] the equilibrium dialysis method,[7)] the potentiometric titration method[8)] and the membrane

1) Presented at the 93rd Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April, 1973.
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4) H.B. Kostenbauder, *Amer. Perfum. Cosmet.*, **75**, Jan. 28 (1960).
5) *a*) T. Higuchi and J.L. Lach, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 465 (1954); *b*) D. Guttman and T. Higuchi, *ibid.*, **45**, 659 (1956).
6) *a*) M. Matsumoto and M. Aoki, *Chem. Pharm. Bull.* (Tokyo), **10**, 251 (1962); *b*) F.W. Goodhart and A.N. Martin, *J. Pharm. Sci.*, **51**, 50 (1962).
7) *a*) N.K. Patel and H.B. Kostenbauder, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 289 (1958); *b*) P.P. Deluca and H.B. Kostenbauder, *ibid.*, **49**, 430 (1960); *c*) C.K. Bahal and H.B. Kostenbauder, *ibid.*, **53**, 1027 (1964); *d*) N.K. Patel and N.E. Foss, *ibid.*, **53**, 94 (1964); *e*) S.M. Blaug and A.G. Rich, *ibid.*, **54**, 30 (1965).
8) *a*) W.P. Evans, *J. Pharm. Pharmacol.*, **16**, 323 (1964); *b*) A.G. Mitchell and K.F. Brown, *ibid.*, **18**, 115 (1966); *c*) M. Donbrow and C.T. Rhodes, *J. Chem. Soc.*, **1964**, 6166.

osmometry method.[9] In our previous work,[3d] an ultrafiltration method for measuring the free preservative in the aqueous phase of the oil-in-water emulsion has been presented, and it has been shown that the ultrafiltration technique using the Diaflo membrane is applicable to study the binding characteristics for preservatives-surfactants interaction.

In this paper, the interaction between methyl *p*-hydroxybenzoate (MP) and polyoxyethylene dodecyl ether (PDE) is examined by means of ultrafiltration technique and nuclear magnetic resonance spectrometry. Moreover the location of the solubilizate within the micelle is discussed.

Experimental

Material——Methyl *p*-hydroxybenzoate (MP), polyoxyethylene (8)[10] dodecyl ether (PDE-8) and the Diaflo membrane, UM-10, 43 mmϕ were as described previously.[3d] Polyoxyethylene (10, 15, 20, 30 and 50)[10] dodecyl ethers (PDE-10, -15, -20, -30 and -50) were of commercial grade and supplied by Nihon Emulsion Co., Tokyo. Deuterium oxide had a purity of 99.9%.

Ultrafiltration and Quantitative Analysis——The technique employed was essentially the same as that described in the previous work.[3d] Ultrafiltration at temperature of 25° was used to separate the free form of MP. The ultrafiltrate was analyzed spectrophotometrically at wavelength of 256 nm. The pH values of the sample solutions in the studies were recorded at the end of the ultrafiltration, with no appreciable change noted.

Nuclear Magnetic Resonance (NMR)——The NMR spectra of PDE-8, alone and in the presence of MP, were obtained at 100 MHz in deuterium oxide using a Varian HA-100 high resolution spectrometer. Tetramethyl silane, in a capillary tube, was used as an external reference. Chemical shifts were measured in Hz and the precision of measurements was ± 0.5 Hz for broad peaks such as that for polyoxyethylene protons in the presence of relatively high concentration of MP.

Result and Discussion

Data Treated as a Partition Phenomenon

The theory behind the partitioning of a preservative in an aqueous surfactant solution system has been described by many authors. McBain and Hutchinson[11] have suggested that it is convenient to treat the micelle as a pseudo-phase and to regard solubilization as the partition of solubilizate between water and the micellar phase. The solubilizate in the micelle is therefore to be considered as being quite separate and distinct from that portion which is in free solution.

For the partition of the unionized preservative between the micelles and the aqueous phase, a partition coefficient, K_m, is given by

$$K_m = \frac{D_b/V_m}{D_f/V_a} \tag{1}$$

where D_b is the amount of preservative in the micellar phase, D_f is the amount of preservative in the aqueous phase, V_m is the volume of the micellar phase and V_a is the volume of the aqueous phase. The value of D_f/V_a means the concentration of preservative in the aqueous phase and can be determined directly by analyzing the filtrate obtained from the ultrafiltration. While, the value of V_m is generally not known but is considered to be proportional to the amount of surfactant, S, over a limited concentration range, thus

$$K_m = \frac{D_b/k' \cdot S}{D_f/V_a} \tag{2}$$

or

$$K_m \cdot k' = K'_m = \frac{D_b/S}{D_f/V_a} = \frac{[D_b]/[S]}{[D_f]/100} \tag{3}$$

9) D. Attwood, P.H. Elworthy and S.B. Kayne, *J. Pharm. Pharmacol.*, **23**, *Suppl.*, 77S (1971).
10) The number in parentheses denoted the nominal number of oxyethylene units per molecule.
11) M.E.L. McBain and E. Hutchinson, "Solubilization and Related Phenomena," Academic Press, New York, N.Y., 1955, p. 75.

where k' is a proportional constant, K'_m is the product of K_m and k', $[D_b]$ is the concentration of micellar preservative in the total solution, $[S]$ is the concentration of surfactant in the total solution, $[D_f]$ is the concentration of preservative in the aqueous phase and all concentrations are expressed in per cent. Strictly the amount of surfactant should be the amount of micelles, but the critical micelle concentration of PDE-15 is about 0.005% and is low enough for a practical viewpoint to be neglected. In this experiment, the amount of micelles was expressed in terms of the amount of surfactant. K'_m is the quantity usually calculated as the apparent partition coefficient.[12] The value of $[D_b]$ is calculated from the total concentration of preservative in the solution, $[D_t]$, $[D_f]$ and $[S]$,

$$[D_b] = [D_t] - [D_f]\left(1 - \frac{[S]}{100}\right) \tag{4}$$

The data and the apparent partition coefficients obtained from the ultrafiltration experiments for MP in PDE-15 solutions are shown in Table I where the saturation ratio is given by $[D_f]/0.22\%$ (solubility of MP in water). As the pH values of sample solutions ranging from 5.0 to 5.6 were far below the pK_a of MP, all of the MP molecules were considered to be in undissociated form in this study. Values of K'_m increase markedly as the saturation ratio reduces, although these are constant for different concentration of the surfactant at a given value of the saturation ratio. These results suggest that the distribution of MP between the micelles and the aqueous phase does not obey the simple partition law. The preservatives distribute more easily to the micelles at low saturation ratio than at high ratio. Under these circumstances a partition coefficient has little meaning. Donbrow[13] studied potentiometric titration to investigate the solubilization mechanism for benzoic acid-cetomacrogol system and found that the solubilization process of benzoic acid was not governed by the partition law. Mitchell[8b] also reported the interaction of benzoic acid and chloroxylenol with cetomacrogol, and observed the variations of apparent partition coefficients with free preservative concentrations.

Table I. Apparent Partition Coefficient for MP between Micelles and Aqueous Phase of PDE-15 Solution

Saturation ratio	% of surfactant in total solution $[S]$	% of total MP in total solution $[D_t]$	% of free MP in aqueous phase $[D_f]$	% of bound MP in total solution $[D_b]$	Apparent partition coefficient K'_m
1	1	0.390	0.220	0.172	78.2
	3	0.696	0.210	0.492	78.1
	6	1.15	0.206	0.956	77.3
0.85	3	0.618	0.189	0.435	76.7
0.75	3	0.553	0.165	0.393	79.4
0.45	1	0.191	0.104	0.088	84.6
	3	0.342	0.098	0.247	84.0
	6	0.566	0.094	0.478	84.7
0.25	3	0.200	0.052	0.150	96.2
0.15	3	0.135	0.033	0.103	104
	6	0.237	0.033	0.206	104
0.12	3	0.103	0.026	0.078	100
0.07	3	0.070	0.016	0.054	113
0.04	3	0.035	0.0078	0.0274	117
0.016	1	0.0079	0.0036	0.0043	119
0.013	3	0.0137	0.0029	0.0109	125
	6	0.0240	0.0029	0.0213	122
0.007	3	0.0069	0.0015	0.0054	127

12) K.F. Brown and M.J. Crooks, *Pharm. Acta Helv.*, **48**, 494 (1973).
13) M. Donbrow, P. Molyneux, and C.T. Rhodes, *J. Chem. Soc.* (*A*), **1967**, 561.

Many authors[7a,c,14] have expressed the ratio of the total preservative concentration to the concentration of the free form in the aqueous phase, as a function of the surfactant concentration, thus,

$$\frac{[D_t]}{[D_f]} = 1 + k\cdot[S] \qquad (5)$$

Plots of this type are normally presented as a single straight line and the slope of the line, k, is considered as the binding capacity of the surfactant. The data for MP in PDE-15 solutions shown in Table I are plotted in this manner in Fig. 1. The ratio, $[D_t]/[D_f]$, is a function not only of the surfactant concentration but also of the saturation ratio. The slope, k, decreases with increasing the saturation ratio and the lowest limiting slope corresponds to data studied by the solubility method. As has been discussed by Kazmi,[15] Eq. (5) can be rearranged into the same form as Eq. (3). Although many investigators have reported a fit of data to Eq. (5), the constant does not permit any assumptions to characterize the interaction.

Data Treated as a Binding Phenomenon

Some authors[8c,13] have shown that the binding of benzoic acid by cetomacrogol is governed by the Langmuir isotherm suggesting that the mechanism of interaction between solubilizate and surfactant is one of adsorption on the surface of the micelle or some other site within the micelle. This type of interaction can be expressed according to the reciprocal form of the equation,

$$\frac{1}{r} = \frac{1}{n} + \frac{1}{n\cdot K\cdot[D_f]} \qquad (6)$$

where r is $[D_b]/[S]$, n and K are constants meaning the maximum number of binding sites on

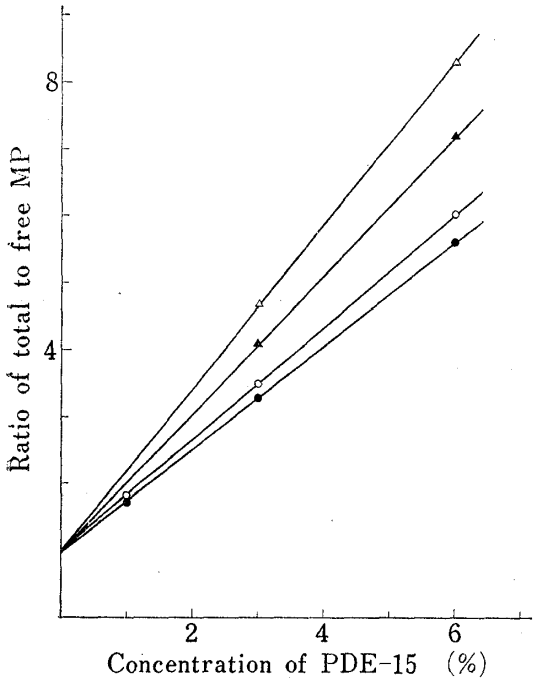

Fig. 1. Ratio of Total to Free MP as a Function of PDE-15 Concentration

saturation ratio; —●—: 1, —○—: 0.45, —▲—: 0.15, —△—: 0.013

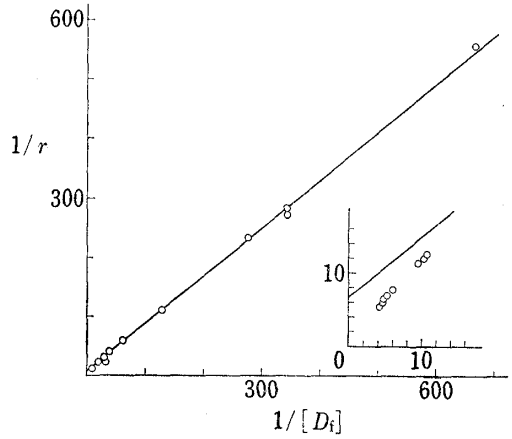

Fig. 2. Langmuir-type Plots for the Interaction of MP with PDE-15

Data shown in Table I were plotted according to Eq. (6).

14) *a*) N.K. Patel and N.E. Foss, *J. Pharm. Sci.*, **54**, 1495 (1965); *b*) J.H. Collett and R. Withington, *J. Pharm. Pharmacol.*, **24**, 211 (1971).
15) S.J.A. Kazmi and A.G. Mitchell, *J. Pharm. Pharmacol.*, **23**, 482 (1971).

the micelle and the association constant respectively. The data for the interaction of MP with PDE-15 shown in Table I are plotted according to Eq. (6) in Fig. 2. The binding of MP by the micelle of PDE-15 seems to obey the form of the Langmuir isotherm at low concentrations of the free preservative, whereas, at high concentrations of the free preservative, data do not fit to a straight line and point to the origin indicating the value of n becomes infinite. An infinitely large value of n appears to show evidence of partitioning mechanism of interaction in a definite range of preservative concentrations near the saturation point. Graphical treatment of data according to Eq. (6) heavily weights those experimental points which are obtained at low concentration of the free preservative and may lead to misinterpretations concerning behaviors at high concentration of the free preservative.

Eq. (6) can be rewritten to yield the Scatchard plot,

$$\frac{r}{[D_f]} = n \cdot K - r \cdot K \tag{7}$$

This plot gives an unbiased weight to each point on the curve and does not suffer from the disadvantage mentioned above. Fig. 3 shows the Scatchard plots of the interaction of MP with PDE having a variety of numbers of oxyethylene units in the homogeneous chain, which include polyoxyethylene (15) derivative. Essentially the same pattern was obtained for the homogeneous surfactants, and as Kazmi[15] reported the interaction of preservatives with cetomacrogol, the plots were curved. This suggests the existence of more than one class of binding sites. Because nonionic micelles have loci of variable polarity, it might be anticipated that the loci would have different affinities for the preservative. Curved Scatchard plots were resolved using a modified method of Hart[16] to obtain the binding parameters. A multiple linear regression technique was incorporated into the procedure and a programmable computer, JEC-5 Spectrum Computer, was used. The binding data can be adequately represented as an interaction with two classes of sites and the binding parameters calculated are listed in Table II. In this case, the interaction was characterized according to Eq. (8).

$$r = \frac{[D_b]}{[S]} = \frac{n_1 \cdot K_1 \cdot [D_f]}{1 + K_1 \cdot [D_f]} + \frac{n_2 \cdot K_2 \cdot [D_f]}{1 + K_2 \cdot [D_f]} \tag{8}$$

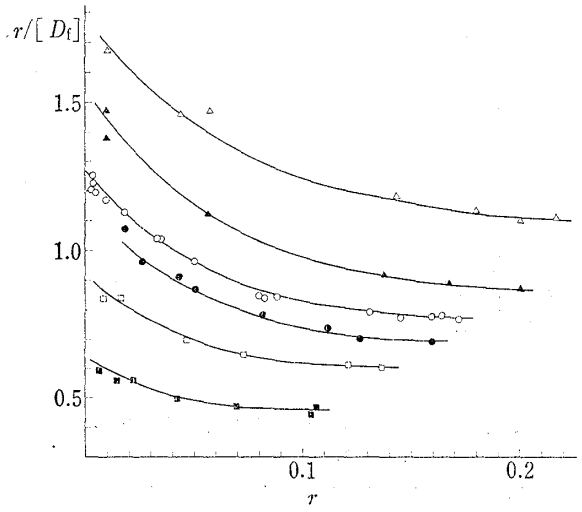

Fig. 3. Scatchard Plots for the Interaction of MP with PDE

—△—: PDE-8, —▲—: PDE-10,
—○—: PDE-15, —●—: PDE-20,
—□—: PDE-30, —■—: PDE-50

The primary class of binding sites has a considerably high affinity but a low capacity for the preservative, while the secondary class of sites has a lower affinity and a greater capacity as indicated by the binding parameters. Such analysis reveals that the contributions of the secondary binding sites to total binding becomes significant at high ratios of preservative to surfactant. If the interaction is assessed only over a limited range of the preservative-surfactant ratios, such secondary class of sites may be overlooked and the binding affinity at primary sites may be underestimated. Therefore, to determine binding parameters accurately, it is necessary to study the binding over a wider range of the preservative-surfactant ratio than is encountered practically.

Donbrow and co-workers[13] have examined the interaction of benzoic acid with the

16) H.E. Hart, *Bull. Math. Biophys.*, **27**, 87 (1965).

TABLE II. Binding Parameters for the Interaction of MP with PDE at 25°

Surfactant	n_1	K_1 (liter/mole)	n_2	K_2 (liter/mole)
PDE- 8	0.121	356	14.0	3.86
PDE-10	0.121	425	16.9	2.92
PDE-15	0.124	410	20.6	2.92
PDE-20	0.143	495	21.3	3.24
PDE-30	0.126	611	69.1	1.22
PDE-50	0.122	357	27.4	3.93

cetomacrogol micelle and discussed the results in terms of the adsorption of all solubilizate molecules at the interface of the hydrocarbon core and oxyethylene mantle of the micelle. Recently, Crooks and Brown[17] have reported the binding of preservatives to cetomacrogol and represented a model of two distinct classes of binding sites in the micelle suggesting that preservatives are solubilized in both the oxyethylene mantle and the hydrocarbon core. Although, by inspecting carefully the binding parameters in this study, it could be thought that the primary class of sites was the form of the Langmuir isotherm and the solubilization involved the binding to the definite site in the micelle. While the values of K_2 were very small and n_2 were exceedingly large, consequently the secondary process seemed to be a non-specific interaction of immense binding capacity which was similar to a simple partitioning mechanism. Since $[D_f] <$ 0.0145 mole/liter and $K_2 \cdot [D_f] \ll 1$, Eq. (8) could be written

$$r = \frac{[D_b]}{[S]} = \frac{n_1 \cdot K_1 \cdot [D_f]}{1 + K_1 \cdot [D_f]} + n_2 \cdot K_2 \cdot [D_f] \qquad (9)$$

The product of n_2 and K_2 would correspond to an apparent partition coefficient concerning the secondary class of sites.

NMR Studies

The interaction occurring between MP and PDE causes a change of environment for both MP and nonionic surfactant molecules. Such changes in environment can be followed by ob-

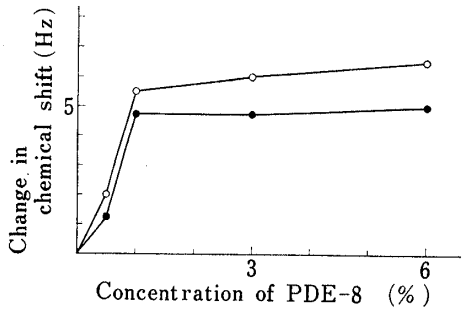

Fig. 4. Changes in Chemical Shift of Ring Protons and Methyl Protons of MP in the Presence of Varying Concentration of PDE-8

MP concentration: 0.3% in the total solution,
—○—: methyl protons,
—●—: *o*- and *m*-protons

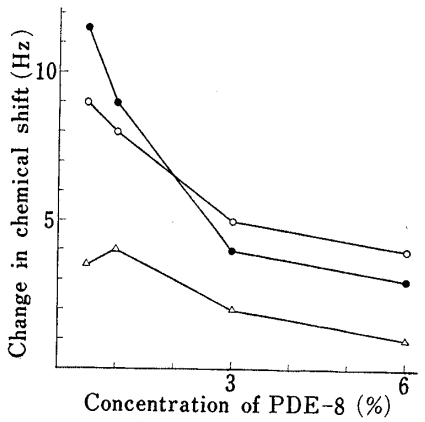

Fig. 5. Changes in Chemical Shift of PDE-8 Protons with Varying Concentration of PDE-8 in the Presence of MP

MP concentration: 0.3% in the total solution,
—●—: polyoxyethylene protons,
—○—: methylene protons,
—△—: methyl protons

17) M.J. Crooks and K.F. Brown, *J. Pharm. Pharmacol.*, **26**, 235 (1974).

serving chemical shifts in the NMR spectra of the components. Fig. 4 shows the change in chemical shifts for both the aromatic ring protons and the ester-linked methyl protons of MP in the presence of increasing concentrations of PDE-8, indicating a change to a less-polar environment as the ratio of micellar MP to free MP increases.

It has been stated[8c,13,18] that the preservative molecules may exist in one or more of three different loci of the micelles; (a) within the hydrophobic core, (b) within the hydrophilic mantle and (c) at the interface of these two loci. The NMR spectra may provide data to suggest the solubilization location in the micelles. The characteristic resonance lines of aqueous PDE-8 also shift upfield in the presence of MP. The upfield shifts for both the polyoxyethylene protons and the alkyl protons with respect to the signals from the surfactant solution are illustrated in Fig. 5, with varying concentration of surfactant in the presence of 0.3% MP.

The greater shift for the methylene protons than that for the polyoxyethylene and methyl protons at high concentration of surfactant, and hence at low ratios of MP to surfactant, shows that the MP molecules accumulate preferentially in or near the hydrocarbon core. It can be considered that the hydrophilic part of the MP molecule may be associated with energetically favorable aqueous environments at the core-mantle junction of the micelles, while the hydrophobic part may contact with the hydrocarbon core. It therefore appears that MP is situated at the oxyethylene-hydrocarbon junction. The very low solubility of MP in dodecane supports this assumption rather than the solubilization into the hydrophobic core. Eriksson[19] has reported that even benzene and its nonpolar derivatives are located primarily at or near the hydrophilic-hydrophobic interface of the micelles. This process corresponds well to the primary class of sites represented by the Langmuir isotherm. At low concentration of surfactant, and therefore relatively high ratios of MP to surfactant, the upfield changes of chemical shift of the polyoxyethylene protons are larger than that of the alkyl protons, suggesting that the site of interaction is different from that at high concentration of surfactant. This finding indicates that the MP molecules are associated with the polyoxyethylene region at high MP to surfactant ratio. This conclusion is supported by the relatively great broadening of the polyoxyethylene peak compared with the alkyl peak. Although many investigators have reported the location of partially polar solubilizates in polyoxyethylene type micelles, there is remarkable discrepancy in the literature as to the contribution of either the mantle or the core of micelles. These differences in results may be ascribed to the lack of consideration on the ratios of solubilizate to surfactant. From the results of this study it is concluded that the most likely locations of the solubilized MP is at the interface of the hydrocarbon core and the polyoxyethylene mantle of the micelle, and also in the polyoxyethylene mantle of the micelle. Both locations could also allow formation of a hydrogen bonded complex between the acidic hydrogen atom of MP and the ether oxygen atom of polyoxyethylene.

Acknowledgement The authors express their sincere thanks to Dr. S. Tatsuoka and Dr. H. Mima for permission and encouragement to carry out this work.

18) *a*) M. Donbrow and C.T. Rhodes, *J. Pharm. Pharmacol.*, **18**, 424 (1966); *b*) J.J. Jacobs, R.A. Anderson, and T.R. Watson, *ibid.*, **23**, 786 (1971); *c*) T.C. Corby and P.H. Elworthy, *ibid.*, **23**, *Suppl.*, 49S (1971); *d*) P. Mukerjee, *J. Pharm. Sci.*, **60**, 1528 (1971).

19) J.C. Eriksson and G. Gillberg, *Acta Chem. Scand.*, **20**, 2019 (1966).