ester group, i.e. methyl, ethyl, propyl, and butyl. Moreover, it is understood that the ability of solubilization is dependent upon the property of the surfactant, particularly upon the chemical structure of its lipophilic group. Therefore, degree of inactivating effect on Parabens differs according to the type of surfactants. e.g., TO-, BO-, and NP-type.

The results obtained in the present series of experiments give a reasonable support to the conclusion that the antifungal activity of *p*-hydroxybenzoic acid ester in the presence of a surfactant is represented only by what is present in aqueous phase outside the micelle.

(Received March 6, 1961)

UDC 615.778.474-014 : 661.185

40. Mitsuo Matsumoto and Masaru Aoki : Application of Surface Active Agents in Pharmaceutical Preparations. XI.[1] Inactivation of *p*-Hydroxybenzoic Acid Esters in the Solubilized Solution of Oily Substances.

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The preceding paper[1] presented the data demonstrating that the antifungal activity of *p*-hydroxybenzoic acid esters in the presence of a nonionic surfactant is caused only by what is present in aqueous phase outside the micelle. The degree of inactivation of *p*-hydroxybenzoic acid esters in the presence of polyethylene glycols or different series of nonionic surfactants having different molar numbers of condensed ethylene oxide was compared and discussed with reference to solubilizing ability. It was suggested that the degree of partition of *p*-hydroxybenzoic acid ester between micellar and nonmicellar phases was not only dependent upon the hydrophilic property of the ester but also upon the chemical structure and balance of hydrophilic and lipophilic affinity of the surfactant.

In regard to the change of effective preservative levels of *p*-hydroxybenzoic acid esters according to the type of preparations, e.g. cream, lotion, etc., Sokol[2] cited a tabulation prepared by Suess.[3] On the basis of this, Sokol suggested that the greater the molecular weight of the ester in the alkyl series is, the smaller becomes the quantity recommended for preservation of any of these types of pharmaceutical preparations. At the same time, however, it was understood that the amount of *p*-hydroxybenzoic acid ester to be incorporated for preservation increased with an increase of oil content in a preparation.

So far, few discussions have been made on individual effect of surfactants and the amount of oils upon the effect of incorporated preservatives.

In the experiments employing Hydrophilic Ointment (J.P. VI and U.S.P. XV) and similar emulsions,[4] it was found that the amount of *p*-hydroxybenzoic acid ester required for preservation was dependent not only on the nature of the ester used but also on the nature of components in oil and water phase.

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In the present work, an attempt was made to investigate the changes of effective preservative levels of *p*-hydroxybenzoic acid ester which was solubilized into a preparation containing an oil phase. In connection with the results obtained in the previous experiments, the surfactants used were selected on the following consideration. It was expected that when oily substances were added to the aqueous solution of a surfactant they would be taken into the lipophilic part of the micelle formed by the surfactant. Therefore, in the case of incorporation of aliphatic compounds, such as liquid paraffin or vegetable oils, BO-type surfactants*[2] (polyoxyethylene oleyl ether) were employed, while NP-type surfactants*[2] (polyoxyethylene nonylphenyl ether), which have a benzene ring as the lipophilic part, were used for the incorporation of xylene. Furthermore, the ratio of hydrophilic to lipophilic affinity of a surfactant had a close relation to its solubilizing ability and was subject to effective concentration on *p*-hydroxybenzoic acid ester. In the present experiment, however, used a series of surfactants were selected from those used previously, which have an appropriate structure for solubilizing oily substances.

Experimental

Materials

***p*-Hydroxybenzoic Acid Esters**: Usually recrystallized from benzene. Methyl *p*-hydroxybenzoate, m.p. 127°, propyl *p*-hydroxybenzoate, m.p. 98°.

Surfactants: A series of surfactants were selected from those employed in the previous experiment,[1] having an appropriate structure for solubilizing oily substances. They were specifically synthesized at Nippon Surfactant Co.

BO-type (polyoxyethylene (−*x* mole) oleyl ether): BO-8 mole, BO-10 mole and BO-12 mole.

NP-type (polyoxyethylene (−*x* mole) nonyl phenyl ether): NP-12 mole

Oily Substances: Liquid paraffin, Carnation oil #35 (Sonneborne Research Co.); isopropyl myristate, IPM-Extra (Nippon Surfactant Co.); lauryl alcohol (Nikko Co.); olive oil, J.P. VI; butyl phthalate and xylene, (special grade, Japan Industrial Standards (JIS) Reagents).

Test Organism: *Aspergillus niger*, a laboratory strain of the Research Institute for Microbial Diseases, Osaka University.

Media: the same composition as that employed in J.P. VI (i.e. a fluid Subouraud medium containing 1% of peptone and 2% of hydrous glucose). A five-fold concentrated medium employed as a basic working broth for antifungal activity test.

Method BO-type surfactants were found most favorable for the solubilization of liquid paraffin, IPM, lauryl alcohol, olive oil, or their mixtures; NP-type surfactants for butyl phthalate or xylene for the same reason. In each case a surfactant which had a suitable ratio of hydrophilic to lipophilic affinity in solubilizing the oily substances was selected from similar type of surfactants having different molar number of condensed ethylene oxide.

Two series of concentrated working solutions were prepared and sterilized. All subsequent procedures were carried out aseptically; one of the series consisted of 5% of a surfactant and a five-fold concentration of the oily substance to be tested, and the other was prepared by adding the former solution to suitable amount of *p*-hydroxybenzoic acid ester which was quantitatively different. An attempt was made to check its effective concentration in a final culture medium. In studying its effect, lauryl alcohol was tested in the form of a liquid paraffin solution because of difficulty of its solubilization. When used as an oily substance, xylene was added aseptically after an adequate mixing of the concentrated working solutions to avoid evaporation during sterilization process.

To prepare 10 cc. of a final test culture medium, an appropriate amount of the concentrated working solution was mixed so as to make 2 cc., which was mixed with 2 cc. of a five-fold concentrated medium, followed by addition of sterillized water to make 10 cc. This final culture medium contains a conjectured amount of *p*-hydroxybenzoic acid ester which assures an effective concentration in the presence of a definite amount (0.25～2.0%) of an oily substance and of a surfactant (1%). After being kept at 25° before inoculation, the final culture medium was inoculated with one platinum loop of a standardized spore suspension of *Aspergillus niger* which was prepared as described in the previous paper. Decreased activity of *p*-hydroxybenzoic acid ester was judged by the growth of the mold after an incubation for 10 days at 25° as reported previously. However, in the presence of more than 2% of liquid paraffin or olive oil, some difficulty was encountered in evaluating the

*[2] Synthesized and kindly supplied by the Nippon Surfactant Co., Ltd. Tokyo.

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mold growth because of emulsification. In such a case, the mold growth was evaluated after another 10 days' incubation in comparison with the degree of cloudiness due to the mold growth in a clear final culture medium which was incubated for the same period of time.

Results

An inactivation of *p*-hydroxybenzoic acid esters is observed when they are incorporated into preparations in which oily substances are solubilized. The degree of inactivation is compared with that which is displayed in the presence of the same surfactant. When liquid paraffin is used as an oily substance, little difference is found in the degree of inactivation as compared with that of BO-10 moles alone, but some difference appears according to the kind of the solubilized oily substances employed. In the case of liquid paraffin with the same concentration of BO-10 moles, the inactivating effect for methyl and propyl esters does not differ from that influenced by the surfactant alone even if a ratio of liquid paraffin to surfactant is increased from 1:0.5 to 1:2 (Table I).

If isopropyl myristate is used instead of liquid paraffin, the activity of propyl ester is considerably interfered; the interferance becomes more marked with an increase of the amount of the isopropyl ether employed. As far as methyl ester itself is concerned, however, a similar degree of inactivation is observed irrespective of the amount of the oily substance (Table II).

Table I. Effective Preservative Levels of *p*-Hydroxybenzoic Acid Ester against *Aspergillus niger* in the Presence of Liquid Paraffin solubilized in 1% Solution of BO-10

Liq. paraffin concn. (%) / Preservative	0	0.5	1	1.5	2
Methyl ester	0.150	0.165	0.153	0.153	0.148
Propyl ester	0.170	0.185	0.185	0.180	0.180

Table II. Effective Preservative Levels of *p*-Hydroxybenzoic Acid Ester against *Aspergillus niger* in the Presence of Isopropyl Myristate solubilized in 1% Solution of BO-12

Isopropyl myristate concn. (%) / Preservative	0	0.5	1	1.5	2
Methyl ester	0.145	0.160	0.175	—	0.175
Propyl ester	0.162	0.230	0.275	0.295	0.300

A marked difference is observed in the effect of liquid paraffin and isopropyl myristate upon the activity of *p*-hydroxybenzoic acid esters, especially of propyl ester. It seems to come from the difference between the two oily substances solubilized since one is a fatty acid ester and the other is a hydrocarbon. In order to compare the influence of oily phase upon the activity of a preservative, oily substances which are different in their nature were solubilized and its results are shown in Table III. It is seen that a vegetable oil and fatty alcohol cause stronger effect than mineral oil, showing an increasing intensity as the amount solubilized increases. Propyl ester is also influenced much more than methyl ester.

During the experiment, application of different kinds of oily substances made it necessary to use a few surfactants which differ slightly in their structure. However, the results shown in Table III regarding the control do not seem to indicate that the use of such surfactants brings about much discrepancy in evaluating the experimental results on the influence of oily substances upon the esters.

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Table III. Influence of Solubilized Oily Substance on Preservative Action of *p*-Hydroxybenzoic Acid Esters against *Aspergillus niger*

Oily substance	Concn. of oily (%) subst. incorporaled	Surfactant (1%)	Methyl ester			Propyl ester		
			Required (%)	Ratio[a] A	Ratio[a] B	Required (%)	Ratio[a] A	Ratio[a] B
Liq. Paraffin	1	BO- 8	0.155	1.1	(2.2)	0.195	1.1	(8.8)
Liq. Paraffin	1	BO-10	0.155	1.05	(2.2)	0.185	1.1	(8.4)
Isopropyl myristate	1	BO-10	0.175	1.2	(2.5)	0.265	2.55	(12.0)
Isopropyl myristate	1	BO-12	0.175	1.2	(2.5)	0.265	2.65	(12.0)
10% Lauryl alcohol[b]	1	BO-10	0.155	1.05	(2.2)	0.185	1.1	(8.4)
20% Lauryl alcohol[b]	1	BO-10	0.165	1.1	(2.3)	0.220	1.3	(10.0)
30% Lauryl alcohol[b]	1	BO-10	—	—		0.240	1.4	(10.9)
40% Lauryl alcohol[b]	1	BO-10	0.170	1.15	(2.4)	0.255	1.5	(11.5)
Olive oil	1	BO- 8	0.155	1.1	(2.2)	0.260	1.4	(11.8)
50% Olive oil[b]	1	BO- 8	—	—		0.200	1.1	(9.1)
Xylene	0.5	NP-12	0.155	1.05	(2.2)	0.170	1.1	(7.7)
Xylene	1	NP-12	—	—		0.260	1.7	(11.8)
Dibutyl phthalate	0.5	NP-12	—	—		0.270	1.7	(12.3)
		BO- 8	0.140		(2.0)	0.180		(8.1)
		BO-10	0.150		(2.1)	0.170		(7.7)
		BO-12	0.147		(2.1)	0.160		(7.2)
		NP-12	0.150		(2.1)	0.155		(7.0)
		none	0.070			0.022		

a) Ratio A : Effective concn. in a solubilized solution of oily substance/effective concn. in solution of surfactant.
B : Effective concn. in a solubilized solution of oily substance/effective concn. in agueous solution.

b) Percentage of the oily substance dissolved in liquid paraffin.

Discussion

When oily substances are added to the aqueous solution of a surfactant in the presence of *p*-hydroxybenzoic acid ester, the ester may be easily taken up into the lipophilic part of the micelles, which has more affinity due to the co-existence of an oily substance. This means that the ester enters lipophilic part of the micelle more deeply than when micelles are formed by the surfactant alone. In such a case, as the affinity of oily substances for the ester becomes stronger, the greater amount of the ester is taken up. Hydrocarbons have a weaker affinity for the ester than oily substances such as fatty acid ester, fatty alcohol or triglyceride. Therefore, if liquid paraffin is added, the micelle which consists of the surfactant and liquid paraffin may have little power to take up the ester into the micelle. As it is apparent from Table I, effective concentration of the esters even shows a tendency to decrease as the amount of the liquid paraffin added increases. It may be considered that the decrease resulted from a decrease of the ability to take up the ester into the micelle. In this case, the property of the micelle may be affected by the introduction of liquid paraffin into it.

On the other hand, since isopropyl myristate has a considerable affinity for the ester, especially for propyl estr, an incorporation of the ester accelerates the migration of propyl ester dissolved in water phase into micelles. Hence, in the solubilized system, the activity of propyl *p*-hydroxy benzoate is observed to decrease according to the amount of isopropyl myristate incorporated.

Similar results are obtained, though in different degrees, when lauryl alcohol or olive oil is used. In the case of xylene or butyl phthalate, extreme inactivating effect on propyl ester is observed due to their strong affinity for the ester.

As suggested in the preceding paper,[1] *p*-hydroxybenzoic acid ester in the presence

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of a surfactant was inactivated by decreased concentration of the ester present in the aqueous phase outside the micelle. The degree of partition between the micellar and nonmicellar phases was dependent upon the hydrophilic property of the ester and upon the chemical structure of the surfactant.

These suggestions may be eloquently advocated by the present experimental results. In the presence of oily substances, a solubilized solution exhibits higher inactivation of the ester than when the ester is solubilized in a solution of a surfactant. A marked inactivation is caused in the presence of xylene which does not seem to enhance growth of the mold. These results seem to lead to further consideration that the ester is highly inactivated by being readily admitted into the micelle which exhibits more affinity after being incorporated with xylene.

Hence, the degree of the inactivation of *p*-hydroxybenzoic acid ester is dependent upon the relationship between the properties of the ester, a surfactant, and the oily substance to be incorporated. A similar inactivation will be observed when phenolic substances or other organic preservatives are used. Analogous considerations on all other kinds of preparations containing a surfactant lead to a conclusion that effective action of a medicinal substance may be influenced by a close correlation between their physical and chemical properties of medicinal substances, surfactants, and other oily ingredients.

Summary

Investigations were made on an inactivation of *p*-hydroxybenzoic acid ester when they were incorporated into preparations in which oily substances are solubilized. Oily substances, such as isopropyl myristate, olive oil, lauryl alcohol, xylene and dibutyl phthalate, considerably interferred with the activity of esters dependent upon the amount and the property of oily substances incorporated. Propyl *p*-hydroxybenzoate was found more subject to inactivation than the methyl ester whenever oily substances were employed. It was suggested that the propyl ester was highly inactivated by easy solubilization into the lipophilic part of the micelle which consisted of a surfactant and an oily substance.

(Received March 6, 1961)

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