Drug–Plastic Interactions II: Sorption of *p*-Hydroxybenzoic Acid Esters by Capran Polyamide and *In Vitro* Biologic Activity

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Abstract □ Sorption of methyl- and propylparaben by capran polyamide was determined by means of an equilibrium sorption method. Data are presented to show that the antimicrobial activity of the parabens for *Aspergillus niger*, *Aerobacter aerogenes*, and *Pseudomonas aeruginosa* is diminished in the presence of capran polyamide. The *in vitro* biologic activity of the parabens in the presence of the nylon was shown to be related to the concentration of "free" or the drug in equilibrium with the plastic.

Keyphrases □ *p*-Hydroxybenzoic acid ester—capran polyamide film, sorption □ Capran polyamide sorption effect—paraben antimicrobial activity □ Antimicrobial activity, parabens—capran polyamide sorption effect

It is generally recognized that the biologic activity of the sorbed drug would be reduced in proportion to the amount sorbed (1–3) and *a priori* observation by Autian *et al.* (4, 5) that preservatives such as *p*-hydroxybenzoic acid esters (parabens) are sorbed by nylon (6) from aqueous solution and hence proportionately inactivated, should be justifiable. In contrast, Myers and Lefebvre reported that nylon in concentrations up to 5% had no adverse effect on the antibacterial activity of benzalkonium chloride (7). Adsorption of drugs by insoluble fillers has been recognized, and Deutsch *et al.* noted that the biologic activity of a vitamin was reduced significantly due to its adsorption on the fillers used in capsules and tablets (8). No direct report could be found on a correlation of drug-plastic sorption data with *in vitro* antimicrobial activity of *p*-hydroxybenzoates.

This report will show that the antibacterial and antifungal activity of methyl- and propylparaben is reduced due to their sorption by capran polyamide.[1] A correlation between the sorption data and *in vitro* biologic activity will also be presented.

EXPERIMENTAL

Materials—Methyl- and propylparaben and the synthetic culture medium were the same as employed in a previous communication (3). Capran polyamide was washed with 50% aqueous ethanol and then rinsed several times with distilled water in order to remove surface contaminants. The nylon samples were dried at 65 ± 1° for 8 hr. and stored in a desiccator until used.

Equilibrium Sorption Studies—The experimental technique has been described (9) in Part I. An accurately weighed sample of capran polyamide [about 1.2 g.; one 10.2 × 15.2-cm. (4 × 6-in.) sheet] was placed in 150-ml. bottles,[2] each containing 90 ml. of varying concentrations of the paraben under study. The bottles were closed tightly using plastic (Bakelite) screw caps lined with thin polyethylene[3] film. Preliminary studies showed that under experimental conditions there was no sorption of the parabens by the polyethylene. The bottles were agitated in a general-purpose shaking bath.[4] The drug content at equilibrium was determined spectrophotometrically (5). The decrease in concentration of the drug in solution was a measure of sorption, and the equilibrium was established at the end of 24 hr. agitation.

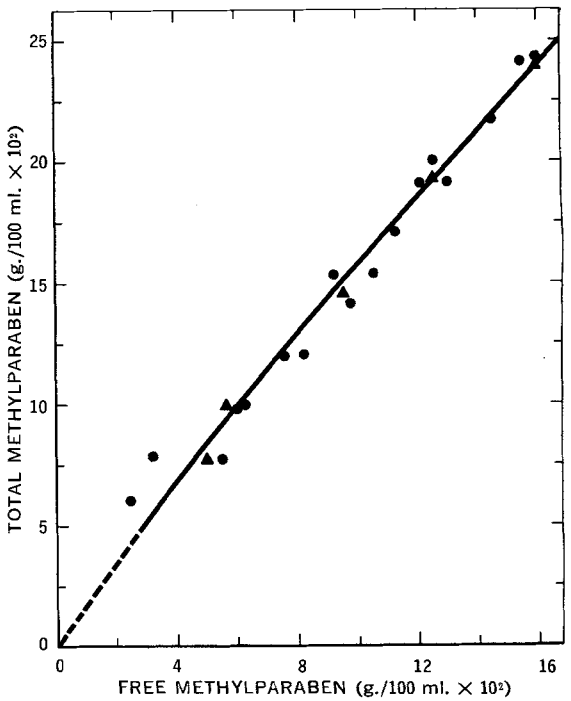

Figure 1—*Sorption of methylparaben by capran polyamide at 30°, showing the total methylparaben concentration in the system as a function of equilibrium or free methylparaben concentration. Key: ●, in culture medium; ▲, in distilled water.*

Microbiological Studies—The general procedure consisted of equilibrating varying concentrations of a paraben in culture medium with a known weight of capran polyamide and determining the inhibitory concentration for one of the organisms. A simple, chemically defined synthetic culture medium as described earlier (3) was used with the exception that 2% dextrose was employed in the place of 5% dextrose. Preliminary experimentation showed that the magnitude of growth of *A. niger* (10), *A. aerogenes* (10), and *Ps. aeruginosa*[5] in the culture medium containing 5% dextrose was about the same as in that containing 2% dextrose. This work also indicated that dextrose, lactose, or sucrose at 2% concentration supported the growth of the above organisms equally well. It was noticed that the lower concentration of sugar was less apt to caramelize at autoclaving temperature. Sterile distilled water was used to prepare all the solutions for microbiological work.

The nylon samples of a definite weight (0.600 g. methylparaben or 0.100 g. propylparaben) were placed in the bottles, each containing 45 ml. of varying known concentrations of the drug. A series of

[1] Nylon 6 film with labeled thickness of 0.0013 cm. (0.0005 in.), lot GHO1077-1-2, supplied through the courtesy of Allied Chemical Corp., Morristown, N. J.

[2] Milk dilution bottles, Fisher Scientific Co., Inc.

[3] Canadian Industries Ltd.

[4] Lab-line Instruments Inc.

[5] Mac-264. The stock culture was grown on slants of nutrient agar and it was supplied by Dr. D. W. Westlake of the Department of Microbiology.

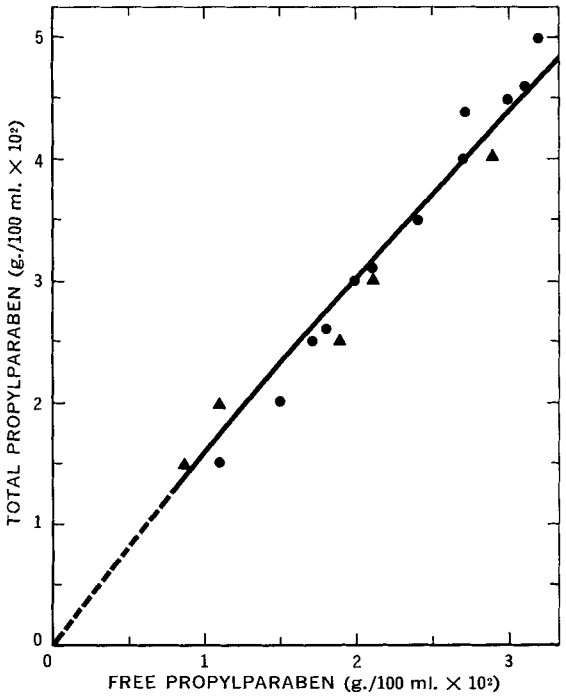

Figure 2—*Sorption of propylparaben by capran polyamide at 30°, showing the total propylparaben concentration in the system as a function of equilibrium or free methylparaben concentration. Key: ●, in culture medium; ▲, in distilled water.*

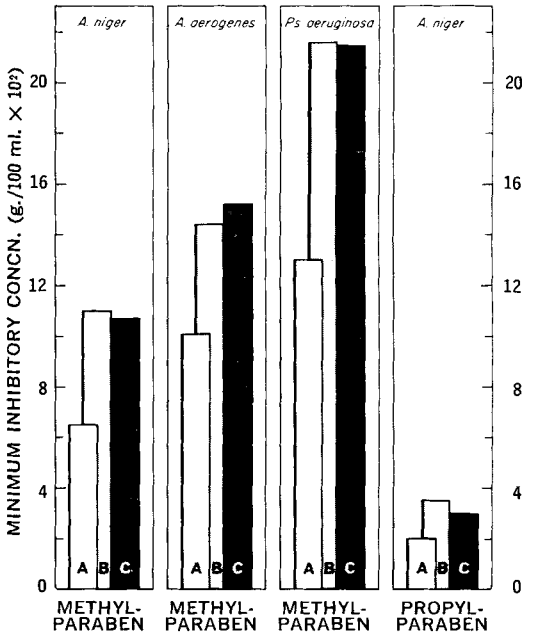

Figure 3—*Comparison of predicted and experimental minimum inhibitory concentrations (M.I.C.'s) of parabens in the presence of capran polyamide. The concentrations of the plastic were 1.33 and 0.222% for methyl- and propylparaben, respectively. Key: A, M.I.C. in absence of capran polyamide; B, experimental M.I.C. in presence of capran polyamide; and C, predicted (from Figs. 1 and 2) M.I.C. in presence of the plastic.*

concentrations of the parabens was prepared in the media, differing by 0.05%. In this manner a paraben solution was prepared containing total paraben as predicted by the procedure described under *Results and Discussion*. Four additional concentrations of the agent were prepared, two of which were lower than the computed value and the other two higher. These solutions were prepared in duplicate. The bottles were closed loosely with plastic caps and sterilized by autoclaving at 15 lb. pressure for 15 min.

The bottles were allowed to attain room temperature in an aseptic area. They were closed tightly and equilibrated by agitation in a water bath at 30° for about 24 hr.

Methyl- and propylparaben were apparently stable under experimental conditions (3). The sorption capacities of the autoclaved and unautoclaved samples of the capran polyamide were the same.

Each of the above bottles was then inoculated with two loopsful of either a spore suspension of the fungus or 48-hr. cultured suspension (3) of the bacteria. In order to maintain a constant concentration of bacteria, the optical density of the bacterial suspension was kept to a value of 0.50 by diluting the contents with sterile medium if necessary. The bottles were incubated at 30° and observed visually for growth in the form of mycelial hyphae in the case of *A. niger* and turbidity in the cases of *A. aerogenes* and *Ps. aeruginosa*.

RESULTS AND DISCUSSION

Sorption of Parabens by Capran Polyamide—The sorption of parabens by nylons has been reported in the literature (4–6). Sorption of methyl- and propylparaben is shown in Figs. 1 and 2, and it is included here for correlation with antimicrobial activity. Similar sorption data have been reported, based on the undergraduate physical pharmacy experimentation (10). Additional results are plotted in Figs. 1–3, showing that there is a fairly good correlation between sorption and microbiological data. The data of Figs. 1 and 2 also illustrate that the sorption of methyl- and propylparaben by capran polyamide in the media of distilled water and synthetic culture is essentially the same. The data are plotted to show that total (sorbed + free) paraben concentration was a function of equilibrium or free paraben concentration. This type of plot is useful for predicting the biologic activity of the drug in the presence of the nylon as explained in the following section.

Method of Prediction of Microbiological Action—If the biologic activity of a paraben can be assumed to be due to equilibrium or free drug in the presence of capran polyamide, and if the minimum inhibitory concentration (M.I.C.) of the paraben in the absence of the plastic is known, then the plots as depicted in Figs. 1 and 2 can be used to predict the total concentration of the drug required for the desired antimicrobial activity. (This assumption supports the theory that the paraben sorbed by capran polyamide is biologically unavailable.) For any desired free concentration, one can obtain the total concentration of the paraben from the ordinates of Figs. 1 and 2. The terms free concentration and M.I.C. in the absence of capran polyamide are assumed to be synonymous for the purpose of prediction.

Inactivation by Capran Polyamide Related to Sorption—The M.I.C.'s of the parabens in the culture medium in the absence of capran polyamide are as follows: methylparaben, 0.065% for *A. niger*, 0.10% for *A. aerogenes*, and 0.13% for *Ps. aeruginosa;* propylparaben, 0.020% for *A. niger*, and 0.034% for *A. aerogenes*. Propylparaben was ineffective in preventing the growth of *Ps. aeruginosa* in a concentration of 0.048%. Based on these M.I.C. values, the inhibitory paraben concentrations in the presence of definite weights of capran polyamide were predicted from Figs. 1 and 2. These predicted values together with experimental inhibitory concentrations in the presence of capran polyamide are portrayed in Fig. 3. It is to be noted that the predicted value (0.052%) of propylparaben for *A. aerogenes* exceeds solubility; therefore, it was not possible to conduct a growth study using this organism in the presence of capran polyamide.

As illustrated in Figs. 1 and 2 the components of the culture medium do not compete for sorption. Thus, any reduction in the biologic activity of the paraben in the presence of the capran polyamide can be attributed to the sorption. Figure 3 demonstrates that there is a good correlation between predicted M.I.C. and experimental antifungal and antibacterial concentrations for the parabens. It is therefore concluded that the plastic-sorbed paraben is devoid of *in vitro* biologic activity.

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Polyamide–Silica Gel Layer Chromatography of Yellow Food Dyes

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Abstract □ The separation of five yellow food dyes and three toxic yellow dyes by mixed polyamide–silica gel thin-layer is described. The method shows good separation and sharp spots. For comparison, the thin-layer chromatography (TLC) on only polyamide and on only kieselguhr is also carried out.

Keyphrases □ Yellow dyes—analysis □ Dyes, yellow—separation, identification □ Polyamide–silica gel chromatography—analysis

The separation of synthetic food dyes on a thin-layer of cellulose (1), silica gel (2), aluminum oxide (3), starch (4), polyamide (5), and paper chromatography (6) has been reported, but none of these techniques gave entirely satisfactory results. Recently, the separation of 11 red food dyes on polyamide (12%)–silica gel G (88%) mixed thin-layers has been successfully applied by Chiang (7). Therefore, further application of this method was tried. In this note, the separation of five yellow food dyes and three toxic yellow dyes (auramine, metanil yellow, and picric acid) by mixed polyamide–silica gel TLC is described. For comparison, the TLC of only polyamide and of only silica gel is also reported.

EXPERIMENTAL

Material—The solvents and chemicals are the reagent grade of Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Preparation of Polyamide–Silica Gel Mixed Layer—Eight grams of polyamide chip (Nylon 6,[1] type 1022B of UBE Industrial Ltd., Osaka, Japan) was dissolved in 80 ml. of 90% formic acid, and then 20 ml. of distilled water was added. After gentle warming (below 40°) and stirring, a homogeneous solution was obtained; this was cooled to the room temperature and 52 g. of silica gel G (E. Merck) was added. Two hundred milliliters of the above-mentioned solution was poured into a dish (14.5 × 19.5 × 2.5 cm.) and a glass plate (12 × 14 × 0.1 cm.) was dipped into it. Both sides of the glass were covered homogeneously. The glass was placed over the dish for 2 min. to let the excess solution drain back. It was then air dried for 3 hr. and heated at 100° for 30 min.

[1] U. S. manufacturer: American Enka Corp.

Table I—Chromatographic Data

No.	Dyes	Solvent I[a] P–S[c]	Solvent I[a] S[d]	Solvent I[a] P[e]	Solvent II[b] P–S	Solvent II[b] S	Solvent II[b] P
1	Naphthol yellow S	0.48	(0.95,	0.37[f])	0.10	(0.74,[f]	0.02)
2	Yellow AB	0.14	(0.62,	0.22)	0.74	(0.97,	0.48)
3	Yellow OB	0.10	(0.52,	0.22)	0.72	(0.97,	0.45)
4	Tartrazine	0.63	(0.98,	0.85)	0.01	(0.37,[f]	0.01)
5	Sunset yellow FCF	0.56	(0.98,	0.66)	0.14	(0.56,[f]	0.08)
6	Metanil yellow	0.28	(0.80,	0.14)	0.47	(0.83,	0.23)
7	Auramine	0.37	(0.71,	0.36)	0.62	(0.71,	0.89)
8	Picric acid	0.40	(0.97,	0.22)	0.53	(0.84,	0.22)
Time required,[g] (min.)		50	30	200	90	70	600

[a] Solvent I: methanol–23% ammonium chloride solution–chloroform (30:20:1.3). [b] Solvent II: isobutanol–ethanol–0.45% sodium chloride solution (3:5:1). [c] P-S, R_f value on polyamide-silica gel mixed layer. [d] S, on silica gel layer. [e] P, on polyamide layer. [f] Tailing. [g] Time required to ascend 10 cm. from origin.

Preparation of Silica Gel Layer—Dilute slurries of silica gel G (45 g. in 100 ml. of water) were sprayed at 2 kg./cm.^{3} pressure from a distance of 20 cm. onto eight sheets of glass plates (12 × 14 cm.) in a horizontal position, then dried at 100° for 30 min. The thickness of the layers was about 250 μ.

Chromatographic Procedure—One microliter of 0.3% alcoholic solution of yellow AB, yellow OB, and auramine, and 0.3% water solution of other dyes was applied to the start line 1.5 cm. from the bottom of the layer, and the plate was developed by ascending techniques. The chamber had been equilibrated with the respective solvent for 30 min. before use.

RESULTS AND DISCUSSION

R_f values obtained with two solvent systems are given in Table I. It has been found that the results obtained by the mixed polyamide–silica gel layers show better separation and sharper spots than that obtained by polyamide and silica gel layers. Also the time required to ascend 10 cm. from origin for the mixed layers is shorter than that for the polyamide layers. Separation mechanism on the mixed layers is based on the formation of hydrogen bonds between