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Keyphrases

Chlorpromazine HCl—quaternary, tertiary forms
Quaternary, tertiary chlorpromazine HCl—activity compared
Motor activity, spontaneous, forced—chlorpromazines
Shock avoidance—chlorpromazines
Hexobarbital sleep-time—chlorpromazines

Interaction of Methyl and Propyl Parabens with Selected Sucrose Esters

By CARLOS VALDEZ, EUGENE I. ISAACSON, and FRANK P. COSGROVE*

The interaction of methyl and propyl *p*-hydroxybenzoates with two sucrose esters, sucrose monotallowate and sucrose monococoate, was studied at different temperatures and concentrations of the sucrose ester by the solubility method. Aspects of the qualitative and quantitative nature of the interaction are reported and a possible mechanism of interaction based on hydrogen and hydrophobic bonding is proposed. Predictive effects on preservative activity due to intermolecular association were tested by microbiologic studies.

NUMEROUS REPORTS in recent years have noted the inactivation of various preservatives in the presence of surface-active agents commonly employed in pharmaceutical preparations (1–11). Examples which have been frequently cited are the inhibition of preservative activity of phenolic compounds in the presence of polyether derivatives of fatty acid esters.

The introduction, in 1956, of a novel series of nonionic surfactants, the fatty acid esters of sucrose, and their proposed use in forming emulsion bases and as dispersing agents in pharmaceutical suspensions warrants a consideration of their possible interaction with preservatives commonly used in such systems. A previous investigation by Blaug and Ebersman (12) using the dialysis method revealed evidence of interaction between fatty acid mono and diesters of sucrose and fatty acid monoesters of propoxylated sucrose and derivatives of benzoic acid. The present study employs the solubility method to investigate the interaction between methyl and propyl *p*-hydroxybenzoates with sucrose monotallowate and sucrose monococoate. The effect of elevation of temperature and change of concentration of surfactant on the association is considered. Microbiologic studies have been designed so as to permit a direct correlation of preservative activity and the degree of binding of the preservative.

EXPERIMENTAL

Materials—Recrystallized methyl *p*-hydroxybenzoate, m.p. 124–126°; recrystallized propyl *p*-hydroxybenzoate, m.p. 95–96°, Eastman Organic Chemicals; sucrose monotallowate; sucrose monococoate, Sucro-Chemical Division, Colonial Sugar Co., Gramercy, La.

Solubility Method—The interaction of methyl and propyl parabens with sucrose monotallowate and sucrose monococoate was studied at different concentrations and temperatures according to the Higuchi and Zuck (13–15) solubility method.

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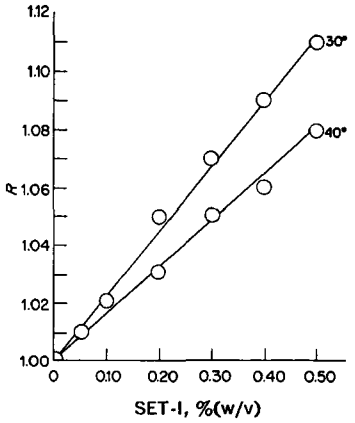

Fig. 1—Ratio R *(total methyl paraben to free methyl paraben) as a function of the concentration of sucrose monotallowate (SET-I) at 30° and 40°. Slope 30°, 0.232; slope 40°, 0.154.*

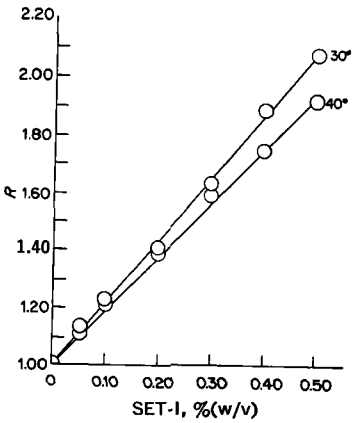

Fig. 3—Ratio R *(total propyl paraben to free propyl paraben) as a function of the concentration of sucrose monotallowate at 30° and 40°. Slope 30°, 2.148; slope 40°, 1.818.*

Quantities (0.5000 g.) of the parabens were weighed and placed in 100-ml. volumetric flasks together with 50 ml. of varying concentrations of the sucrose ester. The stoppered flasks were placed in a mechanical shaker in a constant-temperature bath. The shaking time for each reaction was determined beforehand from equilibration studies. After equilibration, 1-ml. volumetric pipets, the tips wrapped with Whatman No. 1 filter paper, were used to remove aliquot quantities free from excess solid paraben. After appropriate dilution, samples were assayed for paraben content using the spectrophotometer (Beckman DB) at a wavelength of 255 mμ.

Interaction of Methyl Paraben with Sucrose Esters—Data obtained in this study were plotted so as to show the value R, the ratio of total paraben to unbound paraben, as a function of the concentration of the sucrose ester. The degree of association between the reactants can be seen from the diagrams (Figs. 1–4).

A study of Figs. 1 and 2 indicates that the solubility of methyl paraben at 30° increases with an increase in macromolecular concentration in the range of 0.05–0.50% surfactant. An almost identical increase in paraben solubility is noted in the sucrose monotallowate and sucrose monococoate solutions, although the surfactants differ in the range of 4–5 carbon atoms in their fatty acid moieties. This increase in solubility is attributable to the formation of a soluble complex which continues to form without reaching a saturation point in the range studied.

Examination of the slope values for similar studies at 40° indicates that at the elevated temperature, the solubility of the parabens diminishes somewhat from that expected with a 10° temperature rise.

Interaction of Propyl Paraben with Sucrose Esters—It is evident that propyl paraben exhibits a greater solubilizing tendency with sucrose monotallowate and sucrose monococoate than was demonstrated by the methyl ester. A greater than twofold increase in the solubility of propyl paraben at 30° is noted in the presence of 0.5% sucrose monotallowate and 0.5% sucrose monococoate solutions. At 40°, the propyl paraben exhibited a similar diminished solubility compared to that shown by the methyl paraben. It may be observed that, in

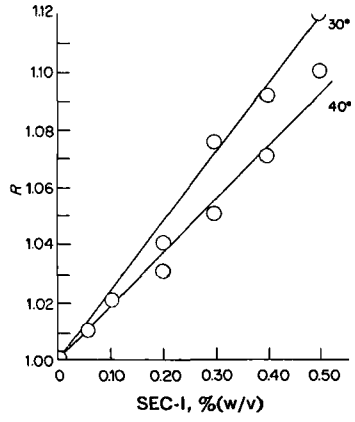

Fig. 2—Ratio R *(total methyl paraben to free methyl paraben) as a function of the concentration of sucrose monococoate (SEC-I) at 30° and 40°. Slope 30°, 0.238; slope 40°, 0.189.*

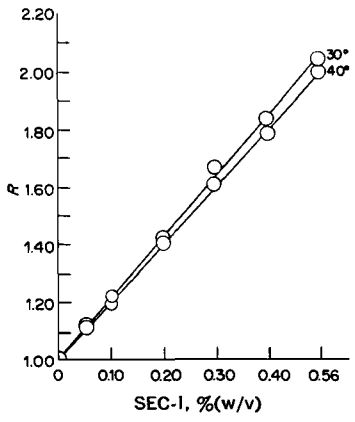

Fig. 4—Ratio R *(total propyl paraben to free propyl paraben) as a function of the concentration of sucrose monococoate at 30° and 40°. Slope 30°, 2.075; slope 40°, 1.969.*

general, the solubility which the parabens exhibit in the presence of the sucrose esters increases with the molecular weight of the parabens. This phenomenon has been reported for the interaction of methyl, ethyl, propyl, and butyl parabens with the polyoxyethylene ester type molecules (16).

DISCUSSION

The Nature of the Paraben-Surfactant Association—The exact nature of the paraben-surfactant association is not completely known. A number of forces have been implicated in other drug-macromolecule associations to account for the experimental data generally obtained (1, 17–21). In aqueous solutions the possibilities exist for hydrogen bonding both in the monomer and in the micellar states. It is possible to visualize that at the dilute concentrations of surfactant employed in this study, paraben molecules may become incorporated within the micelles and associate through a combination of hydrogen and hydrophobic bonding to form a somewhat stable complex.

Binding sites on the hydrophilic portion of the sucrose ester are equivalent to a minimum of 10 ethylene oxide groups based on water-solubility data for the oxygen molecules on the sucrose moiety (22). Results obtained by Blaug and Ebersman (12) and in this study indicate that the macromolecules in question are appreciably less reactive than the polyoxyethylene sorbitan ester series because of the lack of ethylene oxide groups in the sucrose portion of the molecule.

In accordance with the low order of association exhibited by methyl and propyl parabens with sucrose monotallowate and sucrose monococoate, the interaction is postulated to involve primarily hydrogen bonding forces and hydrophobic association. The negative center of the alcoholic hydroxyl of the surfactant is seen as competing for the proton of the phenolic hydroxyl of the paraben more favorably than the carbonyl oxygen of the paraben for the alcoholic hydroxyl hydrogen of the surfactant, although both interactions may occur. The interaction tendencies at 30 and 40° indicate polar forces are operative since these are weakened with an increase in thermal energy. Additionally, however, the small decrease in binding tendency with an increase in reaction temperature of 10° suggests that hydrophobic binding, which is not affected to a great extent by increasing thermal energy (10), is also operative. That hydrophobic binding is operative is supported by the fact that there is a pronounced increase in interaction in going from methyl paraben to the more nonpolar propyl paraben.

MICROBIOLOGIC STUDIES

Microorganisms—The organisms selected for these studies were *Aerobacter aerogenes* and *Escherichia coli*. The strain of *A. aerogenes* exhibited a more suitable growth rate in the simple medium used, however, and was solely used in the study.

Culture Medium—A simple 10% dextrose solution was selected and the strain of *A. aerogenes* was adapted to it. The possibility of an interaction between propyl paraben and the medium was investigated by determining the solubility of the paraben in the 10% dextrose solution at various concentrations of sucrose monotallowate. *R* values obtained did not differ significantly from the aqueous solutions.

Table I—Interaction of Propyl Paraben and Sucrose Monotallowate at 30°

Concn. Set-I (w/v)	Concn. Total Paraben (moles/l. × 10³)	R^a (Biologic)	R^a (Physical Chemical)
0	2.11	1.00	1.00
0.1	2.60	1.23	1.21
0.3	3.52	1.67	1.63
0.5	4.49	2.13	2.08

[a] R = Total paraben/free paraben.

Sterilization—The propyl paraben and sucrose monotallowate were found to be stable to autoclaving procedures used in this study.

Influence of Sucrose Monotallowate on Growth—The presence of sucrose monotallowate in the culture medium exhibited no detectible influence on the growth rate of *A. aerogenes*.

Procedure—Growth studies were carried out in sterile test tubes 25 × 150 mm. (Pyrex). Tubes containing 20 ml. of medium with the desired concentration of sucrose monotallowate and paraben were inoculated with 0.2 ml. of a 72-hr. suspension of *A. aerogenes* grown in plain 10% dextrose medium and standardized to a Klett reading of 100. Tubes were incubated at 30°. Growth was followed using a Klett-Summerson photoelectric colorimeter with a No. 42 blue filter. Turbidity measurements were made after 3 weeks and the minimum inhibitory concentration (M.I.C.) was determined by taking as the inhibitory concentration the range between the lowest paraben concentration not showing growth and the next lowest paraben concentration.

Discussion—Data on the degree of binding of the preservative by the surfactant may be used to predict preservative activity if the assumption is made that preservative activity is a function of the concentration of the free or unbound paraben. On this basis:

$$\text{M.I.C. (predicted)} = R\ \text{(M.I.C.)}$$

at any given concentration studied.

In order to permit a direct correlation of preservative activity in binding, propyl paraben and sucrose monotallowate were studied at 30° and the minimum inhibitory concentrations of propyl paraben were obtained for *A. aerogenes* in a control medium containing no sucrose monotallowate and in media containing several concentrations of sucrose monotallowate.

The method is similar to that used by Pisano and Kostenbauder (7) to study the preservative activity of *p*-hydroxybenzoic acid esters in the presence of polyoxyethylene sorbitan monooleate. Table I illustrates the close correlation of binding data and preservative activity obtained by employing physical chemical and biologic procedures. Microbiologic studies reveal a loss in preservative activity of the propyl paraben in proportion to the degree of binding exhibited by the paraben in the presence of sucrose monotallowate.

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Keyphrases

Parabens, methyl, propyl-sucrose esters—interaction
Solubility method—parabens-sucrose esters interaction
Microbiological analysis—parabens-sucrose esters
UV spectrophotometry—analysis

Identification of Medicinal Barbiturates by Means of Mass Spectrometry

By R. T. COUTTS and R. A. LOCOCK

The mass spectra of the eight barbiturates most commonly prescribed in North America (amobarbital, barbital, butabarbital, mephobarbital, pentobarbital, phenobarbital, secobarbital, and thiopental) and, for comparison purposes, the spectra of butethal (butobarbitone B.P.) and itobarbital were recorded. The weakly acidic material present in capsules or tablets of four different barbiturate-containing products was extracted into ether and the mass spectrum of each extract was recorded. Evidence is presented that it is possible to identify positively individual barbiturates, and mixtures of barbiturates in pharmaceutical dosage forms, by means of mass spectrometry. The mass spectrum of phenacetin (present in one of the capsules studied) is discussed.

THE MASS SPECTRA of various barbiturates of medicinal importance (1) and other barbiturates (2) have been recorded and interpreted. The former study has revealed that under electron impact, barbiturate molecules undergo fragmentations in characteristic manner and the formation of the major ions in their mass spectra has been explained. The data as they are presented, however, do not permit easy differentiation between closely related structures. A positive identification of a particular barbiturate, such as would be desirable in toxicology studies, would not be possible using the reported (1) data.

The present study, therefore, was undertaken to determine whether it was possible to identify positively individual barbiturates, and to see whether mixtures of barbiturates in pharmaceutical dosage forms could be determined qualitatively, using mass spectrometry.

EXPERIMENTAL

Materials—Some of the barbiturates used in this study were purchased from the sources indicated: May and Baker (Canada) Ltd., Montreal (amobarbital, butethal, butabarbital, pentobarbital, and secobarbital); The British Drug Houses Ltd., London (barbital); Merck and Co., Ltd., Montreal (phenobarbital). The remainder were gifts from various drug houses.[1]

Isolation Procedure—*Capsules*—A portion (5 mg.) of the capsule contents was dissolved or suspended in water (2 ml.), concentrated hydrochloric acid (0.1 ml.) was added, and the whole extracted with ether (2 × 5 ml.). The combined ether extracts

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[1] Abbott Laboratories Ltd., Montreal (thiopental); C. E. Frosst and Co., Montreal (Twinbarb capsules); Eli Lilly and Co. (Canada) Ltd., Toronto (Tuinal capsules); A. H. Robins Company of Canada, Ltd., Montreal (Phenaphen capsules); Sandoz Pharmaceuticals, Dorval, Quebec (itobarbital and Plexonal tablets); Winthrop Laboratories, Aurora, Ontario (mephobarbital).