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Influence of Ultraviolet Irradiation on the Surface Activity of Phenothiazine Drugs

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Abstract □ The effect of drugs on the permeability of cell membranes has been related to their surface activity. It was of interest, therefore, to determine the influence of UV irradiation on the surface activity of a series of potential photosensitizers. Solutions of five phenothiazine drugs (1×10^{-4} *M*) were prepared in an acetate buffer. The solutions were exposed to UV irradiation and the surface pressure determined at fixed intervals by the Wilhelmy plate method. Chlorpromazine and prochlorperazine showed an increase in surface pressure during irradiation. Promazine showed no surface activity either before or after irradiation. Trifluprom azine and trifluoperazine exhibited a decrease in surface pressure during irradiation. Both chlorpromazine and prochlorperazine have been clearly established as photosensitizers. The other drugs studied, except for a few isolated instances, have not been reported as photosensitizers. Thus, there appears to be a relationship between an increase in surface activity induced by irradiating a drug and the drug's *in vivo* photosensitizing properties.

Keyphrases □ Phenothiazines, surface activity—UV irradiation effect □ Surface pressure, phenothiazines—UV irradiation effect □ Photosensitization, phenothiazines—surface activity relationship

In previous studies it was established that UV irradiation modified the interaction of some phenothiazine drugs with a lecithin monomolecular film. The chlorine substituted derivatives (chlorpromazine and prochlorperazine) showed a marked increase in reactivity toward the film as the result of irradiation. In contrast, trifluoperazine exhibited a decrease and trifluprom azine and promazine no change in reactivity following irradiation (1).

Of the group of drugs studied, only chlorpromazine and prochlorperazine have been reported to be significantly phototoxic. It was postulated, that the ability of UV-irradiated phenothiazine drugs to interact with a lecithin monolayer may be a measure of their *in vivo* membrane-penetrating and phototoxic properties (1).

It was of interest, therefore, to determine whether these irradiation-induced changes were related to changes in surface activity of the drugs themselves. This paper reports the effect of UV irradiation on the surface pressure of solutions of a series of phenothiazine drugs and several known photo-oxidation products of one of these drugs (chlorpromazine).

EXPERIMENTAL

Materials—The following phenothiazine derivatives were used without further purification: chlorpromazine hydrochloride, chlorpromazine sulfoxide hydrochloride, prochlorperazine, and trifluoperazine dihydrochloride[1]; promazine hydrochloride[2]; trifluprom azine hydrochloride[3]; chlorpromazine *N*-oxide, and 8-hydroxychlorpromazine.[4] The water used was prepared by distillation of deionized water from all glass equipment. All other chemicals were reagent grade.

Apparatus and General Methods—A 0.1 *M* sodium acetate-acetic acid buffer, adjusted to pH 5.9 was used as the solvent in all experiments. The solutions to be irradiated were placed in a trough irradiated either from above or below the surface. In the former case, an UV lamp was secured about 50 mm. above the surface of the solution. In the latter, an UV lamp was positioned under a quartz window set into the bottom of the trough. In both cases the lamps were fitted with filters which screened out radiation below 280 mμ. The temperature of the trough and solution was maintained at 25 ± 0.1° by use of a constant temperature water circulator. Surface pressure, π, (the difference between the surface tension of the buffer solution and that of the drug-buffer solution) was measured by the Wilhelmy plate method (2) using a thin, roughened platinum blade attached to a torsion balance.[5]

The drug solutions were irradiated for periods ranging from 15–25 min., during which time surface pressure was determined periodically. Surface pressure was also determined for varying periods after the irradiation was stopped.

RESULTS AND DISCUSSION

In the first phase of the study 1×10^{-4} *M* solutions of the drugs were irradiated from above the surface for 25-min. periods. Surface pressure was determined at convenient intervals during the irradiation and for 25 min. after the irradiation was stopped. Under these conditions, both chlorpromazine and prochlorperazine exhibited a

[1] Smith Kline & French, Philadelphia, Pa.
[2] Wyeth Laboratories, Philadelphia, Pa.
[3] Squibb Institute for Medical Research, New Brunswick, N. J
[4] National Institutes of Mental Health, Bethesda, Md.
[5] Federal Pacific Electric Company, Newark, N. J.

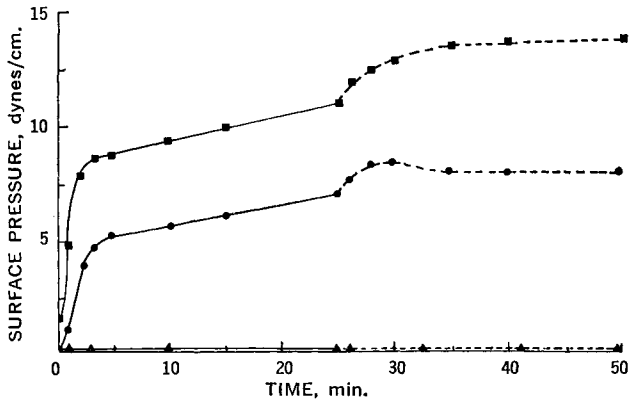

Figure 1—*Surface pressure* versus *time of irradiation of* 1×10^{-4} M *solutions of promazine HCl* (▲); *chlorpromazine HCl* (●); *and prochlorperazine HCl* (■) *at 25°, pH 5.9, and ionic strength 0.1. The dashed line* (- - -) *indicates the period after the irradiation was stopped.*

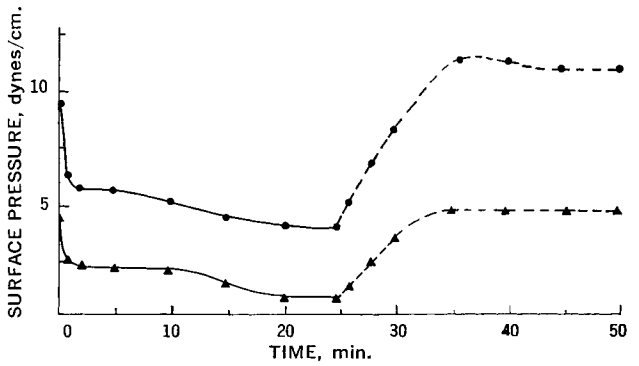

Figure 2—*Surface pressure* versus *time of irradiation of* 1×10^{-4} M *solutions of trifluprομazine HCl* (▲); *and trifluoperazine diHCl* (●) *at 25°, pH 5.9, and ionic strength 0.1. The dashed line* (- - -) *indicates the period after the irradiation was stopped.*

rapid increase in surface pressure over a period of 2–3 min. followed by a slower, secondary change (Fig. 1). Thus, irradiation, in the case of both of the chlorine substituted derivatives, apparently results in the formation of more surface-active compounds. Promazine, an unsubstituted derivative, however, exhibited no change in surface activity even after 25 min. of irradiation (Fig. 1). In contrast, irradiation of the trifluoromethyl derivatives (trifluprομazine and trifluoperazine) resulted in a rapid initial decrease in surface pressure followed by a slower secondary decrease (Fig. 2). When the irradiation was stopped the surface pressure of both of these solutions returned to approximately their original value. The final net result of irradiation was no change in surface pressure in the case of trifluprομazine and about a 2 dynes/cm. increase in the case of trifluoperazine. Thus, it appears that irradiation of the trifluoromethyl derivatives results initially in the formation of a less surface-active species at the surface and in its subsequent desorption from the surface. When the irradiation is stopped, apparently a resorption from the bulk occurs of unchanged material or of a new compound with about the same surface activity as the original compound.

Some of the products of the photo-oxidation of chlorpromazine in the presence of oxygen have been reported to be chlorpromazine sulfoxide, chlorpromazine *N*-oxide, and hydroxychlorpromazine (3). It was of interest to determine whether the formation of these 3 photoproducts during the irradiation of chlorpromazine was responsible for the observed increase in surface pressure. Solutions of each of these compounds (1×10^{-4} *M*) were prepared and surface pressure determined before, during and after irradiation.

In this phase of the study, the solutions were irradiated from below the surface for 15 min. in order to compare the two techniques. The results were essentially the same as that observed previously, except for a slight shift in the time axis due to differences in UV intensity. The results are summarized in Table I.

The chlorpromazine sulfoxide solution exhibited no surface pressure either before or after irradiation. The *N*-oxide, in contrast, exhibited a surface pressure of 5.1 dynes/cm. as opposed to less than 1 dyne/cm. for an equal concentration of chlorpromazine. However, since the irradiation of chlorpromazine resulted in an increase in surface pressure of 9.7 dynes/cm., the formation of *N*-oxide *via* a photooxidation could not account in itself for the total observed increase in surface pressure. Irradiation of the *N*-oxide, however, did result in an additional 6.9 dynes/cm. increase in pressure. Thus, the formation and subsequent photo-oxidation of the *N*-oxide could account for the effect observed on the irradiation of chlorpromazine. A solution of a representative hydroxy derivative (8-hydroxychlorpromazine) prior to irradiation exhibited no significant surface pressure initially. However, on standing the surface pressure of this material increased without irradiation, reaching a maximum value of almost 15 dynes in about 10 min. Concurrent irradiation appeared to have no significant effect on the rate or total change in surface pressure observed with this compound. The formation of the hydroxy derivative *via* photooxidation and its subsequent change in surface activity could be in part responsible for the changes observed during the irradiation of chlorpromazine.

CONCLUSION

The irradiation-induced changes in surface pressure observed in this study parallel those previously reported in the presence of a lecithin film (1). Those drugs that became more surface active after irradiation (chlorpromazine and prochlorperazine) exhibited an increase in interaction with a lecithin film under similar conditions. Those that remained unchanged or decreased in surface activity (promazine, trifluprομazine, and trifluoperazine) behaved in much the same way in the presence of the film.

Apparently then, the irradiation-induced changes in surface activity of these drugs are directly related to, and possibly responsible for, the irradiation-induced changes in reactivity toward a lecithin film.

This suggests that the photoactivated sensitizing phenothiazine drugs do not react directly with cellular components. Instead, they apparently form a new, stable, more surface-active compound capable of inducing a cutaneous reaction, probably *via* changes in cell membrane permeability.

Burckhardt *et al.* (4) and Willis *et al.* (5) demonstrated similar mechanisms of action for the photosensitized cutaneous reactions produced by 1-butyl-3-sulfanilurea and tetrachlorsalicylanilide, respectively. In both these studies it was established that stable photooxidation products, when applied to the skin in the absence of light, elicited a reaction similar to that observed when the original compound was applied and irradiated. Thus, as noted by Willis *et al.* (5) the interaction of unstable, light-induced, free radicals with specific cellular components need not be assumed to be one of the steps in the process as previously postulated (6). Alternatively, it is the interaction of a new, stable photo product with the cells or cell components that produces the observed effects.

In this study, the photoproducts responsible for the change in surface activity, and presumably for the photosensitizing properties, were not identified. However, in the case of chlorpromazine it appears that the formation and subsequent photolysis of the *N*-oxide derivative is in part responsible for the observed increase in surface activity. Formation of 8-hydroxychlorpromazine *via* irradiation, fol-

Table I—The Effect of UV Irradiation on the Surface Pressure, π, in Dynes/cm. of 1×10^{-4} Solutions of Chlorpromazine and Some Oxidation Products

Compd.	π Prior to Irradiation	π Following Irradiation	$\Delta\pi$ Due to Irradiation
Chlorpromazine (CP)	0	9.1	9.1
CP *N*-oxide	5.1	12.0	6.9
CP Sulfoxide	0	0	0
8-Hydroxy CP	0	[a]	[a]

[a] π Changed spontaneously reaching a maximum value of 15 dynes/cm. in about 10 min. Irradiation did not influence this change.

lowed by a spontaneous change in its surface properties, could also account for some of the change observed following irradiation of chlorpromazine. The sulfoxide, at these concentration levels, would not be expected to contribute to this change.

Since all of these compounds are metabolites of chlorpromazine (7), it would appear that the accumulation in the skin of not only the parent compound, but, more importantly, of some of its metabolites, may be responsible for the reported phototoxic reactions. It would be interesting to determine whether a relationship exists between the level of chlorpromazine and its *N*-oxide in the skin and the incidence of phototoxicity.

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Metabolism of the Plant Growth Regulator 2,3,5-Triiodobenzoic Acid in Soybeans

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Abstract □ This study investigates the residues resulting from the treatment of soybean plants with 1-^{14}C-2,3,5-triiodobenzoic acid. TLC was used to characterize and quantitate the residues present in the various samples. Residues of 1-^{14}C-2,3,5-triiodobenzoic acid, 1-^{14}C-2,5-diiodobenzoic acid, and 1-^{14}C-3,5-diiodobenzoic acid were found in the various plant parts and in the harvested seeds. The seeds also contained a large amount of the ^{14}C activity as a hexane-soluble material which was not identified.

Keyphrases □ 2,3,5-Triiodobenzoic acid metabolism—soybeans □ Soybean plants—1-^{14}C-2,3,5-triiodobenzoic acid residues □ TLC—separation, analysis □ Scintillometry—analysis

Reports of an increased soybean yield following the use of the plant growth regulator, 2,3,5-triiodobenzoic acid (I) (1–3) have led to a proposal for widespread use of the compound on soybeans (4). A more recent publication, dealing with the residue properties of 1-^{14}C-2,3,5-triiodobenzoic acid (II) (5), reported that ^{14}C activity was lost rapidly from the plant but that a residue containing ^{14}C was detected in the harvested seeds. The nature of the ^{14}C residue was investigated.

EXPERIMENTAL

Materials and Methods—The chemicals used in this investigation were reagent grade with the exception of the chromatographic standards.

All samples were counted in a liquid scintillation counter[1] with a gain of 18% and a window setting of 50 to 900 units. The scintillation cocktail used consisted of 15 ml. of a 1:1 mixture of toluene and 2-ethoxyethanol with 3.0 g. of PPO[2]/l. of cocktail. All samples were corrected for quenching by the addition of an internal standard.

The synthesis of the 1-^{14}C-2,3,5-triiodobenzoic acid used in this investigation has been described in a previous publication (5). Samples of leaves and stems from the earlier study were frozen and stored for use in the metabolite analysis. The harvested soybeans from the previous study were stored at room temperature.

TLC was used for the metabolite analysis. The chromatograms were prepared in layers that were 250 μ thick using a 1:2 mixture of an adsorbent containing a fluorescent indicator[3] and plain adsorbent.[3] The chromatograms were activated for 1 hr. at 110° immediately prior to use.

Three solvent systems were used for TLC. Solvent System *A* consisted of a 1:10 mixture of propionic acid and petroleum ether (30–60°), which was freshly prepared and placed in a chamber lined with filter paper. Chromatograms were developed in Solvent System *A* after the solvent had reached the top of the filter paper liner (1.5–2.0 hr.).

Solvent System *B* consisted of a 1:2:10 mixture of propionic acid, methanol, and benzene. Freshly prepared solvent was placed in a chamber lined with filter paper and the chamber was allowed to equilibrate for 24 hr. prior to use.

Solvent System *C* consisted of a 3:7 mixture of pyridine and benzene. Solvent System *C* gave better separation when the chromatograph was placed into the solvent chamber immediately after the addition of the solvent.

After all aromatic solvents had evaporated from the developed chromatograms, the compounds were located by placing the chromatogram under UV light. Table I lists the compounds which were used as chromatographic standards, their source, and their R_f values in the above-mentioned solvent systems.

Preparative thin-layer chromatograms were prepared from a

[1] Packard Tricarb, Packard Instrument Co., Inc.

[2] 2,5-Diphenyloxazole.

[3] Adsorbosil-p-1, and Adsorbosil-1, Applied Science Laboratories, Inc.