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Interaction of Doxorubicin with Phospholipid Monolayers

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Abstract □ The energies of interaction of doxorubicin hydrochloride and sodium 1,2,4-trihydroxy-9,10-dioxo-3-anthracenesulfonate with dipalmitoylphosphatidylethanolamine and dipalmitoyllecithin monolayers spread at the air–water interface were estimated from the increase in surface pressure with increasing concentrations of the subphase-injected compound. Their orders of magnitude were consistent with those of the energies of interaction of doxorubicin and acridines with double-stranded DNA, which suggests that the same type of van der Waals forces are operative.

Keyphrases □ Doxorubicin—energy of interaction with phospholipid monolayers at air–water interface □ Phospholipid monolayers—energy of interaction with doxorubicin and substituted anthracenesulfonate at air–water interface □ Monolayers, phospholipid—energy of interaction with doxorubicin and substituted anthracenesulfonate at air–water interface □ Antineoplastic agents—doxorubicin, energy of interaction with phospholipid monolayers at air–water interface

Doxorubicin is an anthracycline glycoside antibiotic formed by the tetracyclic quinoid aglycone doxorubicinone and the amino sugar daunosamine (1–3). Its cationic form shows antitumor activity (4). The anthracyclines are representative of a class of drugs whose pharmacological activity depends on their binding with nucleic acids and the subsequent inhibition of nucleic acid synthesis (5).

The energies of interaction of alkanols with dipalmitoyllecithin and dipalmitoylphosphatidylethanolamine monolayers spread at the air–water interface were recently correlated with their permeabilities across biomembranes and with the partition coefficients between (*a*) red cell membranes and water and (*b*) phospholipid liposomes and water (6). The present study examines the surface activity of doxorubicin and its energy of interaction with phospholipid monolayers spread at the air–water interface.

EXPERIMENTAL

Reagents—Doxorubicin (I) hydrochloride[1] and sodium 1,2,4-trihydroxy-9,10-dioxo-3-anthracenesulfonate[2] (II) were used without further purification. Dipalmitoyllecithin[3] (III), dipalmitoylphosphatidylethanolamine[4] (IV), the hexane[5] used for the preparation of the spreading solutions, and the distilled water used as subphase and for the preparation of the aqueous solutions fulfilled the requirements previously specified (7, 8).

Instruments and Methods—A 9-cm diameter polytef dish, provided with two identical microburets[6] and a polytef-coated stirring bar, was used as a trough. Surface tension was measured with a Wilhelmy platinum plate attached to an electrobalance[7] whose output was fed into a dual-pen recorder[8]. The methods for the measurement of the surface tension of aqueous solutions and of the change of the surface pressure of the phospholipid monolayer as a function of time after the injection of the drug in the subphase already were described (6–8). The criterion of equilibrium was the constancy, ± 0.1 dyne/cm, of the surface pressure increment, $\Delta\pi$, over 30 min. In all injection experiments, the initial surface pressure, π, of the phospholipid monolayer was 5 ± 0.1 dynes/cm and the temperature was 20 ± 1°.

RESULTS

The surface tensions of aqueous solutions (10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} *M*) I hydrochloride and II were equal to the surface tension of the pure distilled water used to prepare the solutions (72.80 dynes/cm) within the limits of experimental error (±0.1 dyne/cm). The pH values of the I hydrochloride solutions were 5.3, 5.5, 5.4, and 5.6, respectively, and those of the II solutions were 4.1, 4.3, 4.8, and 5.0, respectively.

Typical plots of the increment of the surface pressure, $\Delta\pi$ (dynes per centimeter), as a function of time, *t* (minutes), after the injection of I hydrochloride and II beneath III and IV monolayers are given in Fig. 1 for the same final concentration (4.28×10^{16} molecules/cm^3) of the drug injected in the subphase. The kinetics of the processes are similar, but the highest value of the equilibrium surface pressure was found for the injection of I beneath the IV monolayer (Table I). The energies of interaction were estimated from the slopes of the reciprocals of the equilibrium surface pressures, $\Delta\pi_{eq}$ (dynes per centimeter), after the injection against the reciprocals of increasing final concentrations, *n* (molecules per cubic centimeter), of the subphase-injected drug (7, 8). Such energies are given in Table I.

DISCUSSION

In accordance with the Gibbs adsorption equation, the fact that the surface tension of water is not affected by the presence of 10^{-4}–10^{-7} *M* concentrations of I hydrochloride or II indicates that the concentrations of these solutes at the interfacial region are identical with the concentrations of the bulk aqueous solutions in both cases; *i.e.*, no spontaneous adsorption of those molecules takes place at the air–aqueous solution interface between these concentrations.

[1] Adriamycin, Adria Laboratories, Wilmington, Del.
[2] Pfaltz & Bauer, Flushing, N.Y.
[3] Applied Science Laboratories, State College, Pa.
[4] Schwarz-Mann Research Laboratories, Orangeburg, N.Y.
[5] J. H. Baker Chemical Co., Phillipsburg, N.J.
[6] Manostat, New York, N.Y.
[7] Cahn Division, Ventron Instruments Corp., Paramount, Calif.
[8] Leeds Northrup, North Wales, Pa.

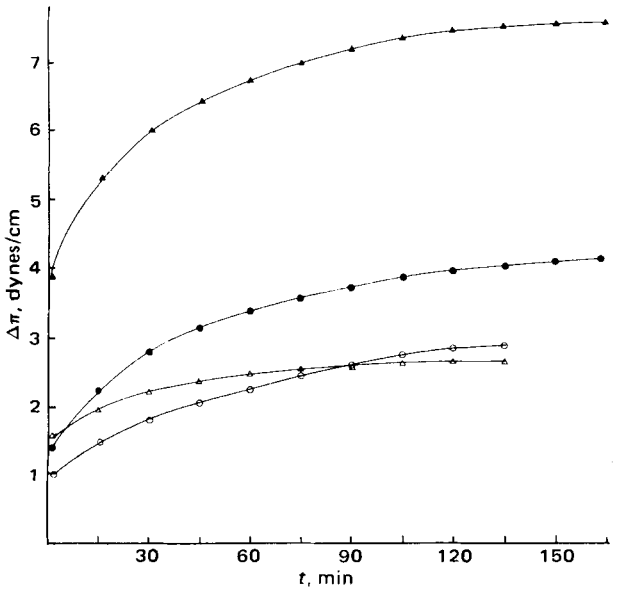

Figure 1—*Typical plots of the change of the surface pressure, $\Delta\pi$, against time, t, after the injection of a final concentration of 4.28 × 10^{16} molecules/cm^3 of I hydrochloride beneath IV (▲), I hydrochloride beneath III (●), II beneath IV (△), and II beneath III (○).*

The protolytic equilibria of I recently were studied by electronic absorption spectroscopy (9). The only species significantly present in the region below pH 7.0 is the singly charged species, with the positive charge located at the amino sugar group. Accordingly, under the experimental conditions (pH 5.3–~5.6), I is positively charged in these aqueous solutions. From the reported (10) pK values of cyclic sulfonic acids, it can be reasonably assumed that II under the experimental conditions (pH 4.1–~5.0) exists in aqueous solutions primarily as the negatively charged form.

Compound IV forms a condensed liquid monolayer. The strong P^-–N^+ electrostatic interaction of the zwitterionic hydrophilic polar groups jointly with the attractive forces between the hydrocarbon chains of neighboring molecules should produce a rigid structuring of the monolayer where any net electrical charge is essentially negligible. The surface area that corresponds to each molecule that forms the monolayer was 40 ± 2 Å^2 at 5 ± 0.1 dynes/cm and 20 ± 1° (8). From the cross-section area of a straight saturated hydrocarbon chain (18–19 Å), the average total number of molecules necessary to form the monolayer under the experimental conditions (10.7 × 10^{15} molecules) (8), and the total surface area of the air–water interface (42.56 cm^2), it can be estimated that the phospholipid molecules occupy between 95.5 and 90.5% of the total surface area, leaving only 4.5–9.5% of free or accessible area of the air–water interface.

Compound III forms a relatively less condensed monolayer at the air–water interface because the shielding effect of the positively charged amino group by the three methyl groups decreases the attractive potential between neighboring molecules (8). The surface area that corresponds to each molecule of the monolayer was 55 ± 2 Å^2 at 20 ± 1° and 5 ± 0.1 dynes/cm, and the average total number of molecules necessary to form the monolayer was 7.7 × 10^{15} molecules (8). In this case, the estimation of the percentage of the total surface area of the air–water interface occupied by the III molecules gives between 65.1 and 68.8%, leaving 34.9–31.2% of free or accessible area.

From literature data on IV (11) and III (12) monolayers, it can be reasonably assumed that the change of the ionic strength (~10^{-4}) resulting from the different final concentrations of the subphase-injected substance will have a negligible effect, if any, on the surface pressure of the phospholipid monolayers.

Theoretical treatments based on the "osmotic approach" and on the concept of "accessible area" have been proposed to explain the penetration of insoluble monolayers by molecules injected in the aqueous subphase (13–15). The accessible area, a, of an insoluble monolayer is defined by (14):

$$a = A - NA_m \qquad \text{(Eq. 1)}$$

where A is the total surface area of the air–water interface, N is the total number of monolayer molecules, and A_m is the area actually occupied by each molecule of the monolayer.

Table I—Surface Pressures of Phospholipid Monolayers and Energies of Interaction after the Injection of I Hydrochloride and II so that the Final Subphase Concentration is *n*

Phospholipid Monolayer	I Hydrochloride			II		
	n, molecules/cm^3	$\Delta\pi_{eq}$, dynes/cm	ψ, kcal/mole	n, molecules/cm^3	$\Delta\pi$, dynes/cm	ψ, kcal/mole
IV	4.28 × 10^{16}	7.7		4.28 × 10^{16}	2.7	
	8.26 × 10^{16}	8.9	7.9	8.26 × 10^{16}	3.6	6.9
	11.96 × 10^{16}	9.7		11.96 × 10^{16}	4.1	
III	4.28 × 10^{16}	4.2		4.28 × 10^{16}	3.0	
	8.26 × 10^{16}	5.4	7.1	8.26 × 10^{16}	4.7	6.4
	11.96 × 10^{16}	6.2		11.96 × 10^{16}	5.8	

The equilibrium penetration is considered as the equilibrium adsorption of the injected molecules at the air–water interface covered with the monolayer, and the adsorption is assumed to occur at the aqueous spaces between monolayer molecules, obeying the equation (12):

$$\Gamma_f = \Gamma_w - A_m\Gamma_w(1/\hat{A}) \qquad \text{(Eq. 2)}$$

where Γ_f and Γ_w are the equilibrium surface concentrations of the injected molecules on a monolayer-covered surface and on a monolayer-free air–water interface, respectively, and $\hat{A} = A/N$ is the area that corresponds to each monolayer molecule.

The increase in the surface pressure, $\Delta\pi$, observed in all cases after the injection of I hydrochloride and II beneath the phospholipid monolayers is in accordance with the osmotic approach and can be considered to be produced by an increased number of molecules at the interface because of the incorporation of the molecules of the injected substance at this surface tension-determining region (13).

The average accessible area of the IV monolayer in the experiments presented here was 7%, and that of the III monolayer was 33%. The results (Table I) indicate that the increase in surface pressure of a IV monolayer produced by the injection of equimolecular amounts of I hydrochloride is, on the average, 64% higher than that produced on a III monolayer. If the increase of the surface pressure of the phospholipid monolayer is reasonably taken as an indication of the extent of adsorption, these results contradict the idea that adsorption at the aqueous spaces between the monolayer molecules is wholly responsible for that increase.

The increase of the surface pressure produced on a IV monolayer by the injection of equimolecular amounts of II is, on the average, 27% lower (Table I) than that produced on a III monolayer. In this case, the results seem to be in accordance with the higher accessible area or aqueous space of the III monolayer. However, the estimated energies of interaction (Table I) are higher in the case of the IV monolayer, which is the more condensed monolayer, *i.e.*, that contains more phospholipid molecules per unit surface area at the same initial surface pressure.

The adsorption of I hydrochloride and II at the aqueous spaces between the monolayer phospholipid molecules is difficult to reconcile, in any case, with the absence of spontaneous adsorption at a monolayer free air–water interface as shown by the independence of the surface tension of the aqueous solutions of both substances with respect to their bulk molar concentrations. Without spontaneous adsorption at the air–water interface, the average statistical distribution of the injected molecules ought to be uniform throughout the whole system. The fact that the surface pressure of the phospholipid monolayer increases steadily after the injection to attain an equilibrium value, $\Delta\pi_{eq}$, seems to indicate an uneven distribution, with part of the injected molecules incorporated at, and restricted to, the surface tension-determining region, possibly through interactions with the phospholipid monolayer molecules.

It was suggested (7, 8) that in the interaction of alkyl sulfates and alkyltrimethylammonium ions with dipalmitoylglycerol, III, and IV monolayers, the first step was the orientation of the polar charged head of the injected surfactant ion toward the attractive centers of the hydrophilic moiety of the molecules that form the monolayer and that the subsequent increase of the surface pressure reflected the subsequent interaction of the hydrocarbon chains with the hydrophobic moiety, mainly through van der Waals forces. When the C_1–C_{14} alkanols interacted with III and IV monolayers, the phospholipid monolayers behaved similarly to ultrathin oil phases (6).

The interaction of I hydrochloride and II with the phospholipid monolayers seems to obey a similar pattern (Fig. 1). The smaller values of the equilibrium surface pressure, $\Delta\pi_{eq}$ (Table I), obtained with equimolecular amounts of II for both monolayers may be related to its smaller

tricyclic hydrophobic moiety as compared with the tetracyclic hydrophobic moiety of I that, in turn, may account for the higher energies of interaction found for I.

Based on the intercalation model to describe the binding of acridines to DNA by insertion between adjacent base pairs (16–18), it recently was proposed (19) that, in the interaction of I with nucleic acids, the positive charge of the amino sugar associates through electrostatic forces with negatively charged phosphate groups of the DNA chain while the tetracyclic hydrophobic moiety, through van der Waals interactions, inserts itself between adjacent base pairs. The binding energy of the acridines to double-stranded DNA is about 6–10 kcal/mole (20). The binding energy of I to double-stranded DNA, recently estimated by using the values of the association constants measured with spectroscopic methods (19), is about 7–12 kcal/mole.

The results presented here seem to indicate that the same type and order of magnitude of van der Waals forces are in effect in the interaction of I and II with phospholipid monolayers.

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^{13}C-NMR Spectroscopy of Tropane Alkaloids

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Abstract □ The natural abundance ^{13}C-NMR spectra of tropine, atropine, scopolamine, cocaine, atropine methonitrate, and *dl*-tropic acid were determined at 22.63 MHz. With the aid of proton decoupling techniques and by comparison with analogous simpler compounds, it was possible to make self-consistent and unambiguous assignments of all carbon resonances for these alkaloids. Some important chemical shift trends were observed and should be useful in the identification of similar systems.

Keyphrases □ Tropane alkaloids, various—^{13}C-NMR spectra determined, carbon resonances assigned □ Alkaloids, various tropane—^{13}C-NMR spectra determined, carbon resonances assigned □ ^{13}C-NMR spectroscopy—various tropane alkaloids, spectra determined, carbon resonances assigned

The proton magnetic resonance (PMR) spectra of alkaloids often are too complex to be useful in the structure elucidation of this large class of naturally occurring compounds. The complexity results from extensive spin–spin coupling among protons, overlap of numerous resonance patterns, line broadening arising from intermolecular association and/or ^{14}N-quadrupolar relaxation effects. Natural abundance ^{13}C-NMR spectroscopy has been especially useful in such cases (1).

The tropane alkaloids have been well characterized by structural and stereochemical investigations (2). However, only one limited ^{13}C-NMR study, concentrating on the nonaromatic part of atropine, has been reported (3). The undertaking of a more detailed study was stimulated during the examination of the stability of tropane alkaloids in organic solvents when, consequently, need arose as to the structure elucidation of closely similar compounds. In addition, the broad acceptance of ^{13}C-NMR spectroscopy as a new powerful tool of structural analysis awaits only the accumulation of chemical shift data on compounds representative of all types of natural products.

This report concerns the application of pulse and Fourier transform ^{13}C-NMR techniques to structure assignments of tropane alkaloids.

EXPERIMENTAL

^{13}C-NMR Spectra—^{13}C-NMR spectra were determined at 22.63 MHz in the Fourier transform mode using a spectrometer[1] interfaced with a computer system[2]. The spectrometer features field stabilization *via* internal deuterium lock. Alkaloidal bases were dissolved in chloroform-*d*, and tropic acid was dissolved in methanol-d_4; either a dimethyl sulfoxide-d_6 or deuterated methanol–deuterium oxide mixture (6:4) was used

[1] Brucker W-90 pulse.
[2] Brucker-Nicolet B-NC-12.