the surface group density as calculated from bulk density, whereas the use of polymeric nonpolar phases introduces other uncertainties. From a practical point of view in evaluating the dispersion force component, γ_S^d, of polar solids using polar liquids, one can use a γ_L^d value obtained with one alkane and relate it to another; however, the use of polymers for this purpose is not appropriate. If one has to determine a γ_L^d for later use with solids, it is probably best, in a practical sense, to use data obtained with paraffin as the standard γ_L^d. The theoretical uncertainties of the entire concept behind γ^d, however, should still be kept in mind.

An earlier study (1) showed that the free energy of solution per methylene group for an homologous series of drugs correlates well with the interfacial tension of the various polar solvents used against tetradecane. Later unpublished results indicate that similar correlations exist for interfacial data obtained with hexane and paraffin. Explanations for these correlations are based upon the concept that this is a measure of the work required to create a cavity of given area in the polar solvent so as to allow a methylene group to enter and interact. From the results of this experiment, additional insight is gained into the major energetic factor contributing to this process. It is seen from the work-of-adhesion data that, regardless of the polar liquid used, the contribution of dispersion forces remains fairly constant. Water is a little lower in each case, possibly because of its smaller molecular size and polarizability relative to the organic solvents or because of some specific structure-forming ability. This suggests that the main difference between the various solvents is the work required to overcome polar–polar interactions.

By using the γ_L^d values obtained from hexane and tetradecane data and Eq. 2, it is possible to calculate γ_L^p for each liquid. This term is directly related to polar group interactions in the solvent. If γ_L^p is plotted *versus* the interfacial tension of that liquid against the corresponding liquid alkane, one can see that the points all fall on a line with a slope of unity or that in all cases γ_L^p is equal to interfacial tension. Thus, it is concluded that, when one sees a correlation with interfacial tension, as observed in the solubility work (1), one is dealing with energetic differences solely associated with the polar portion of the molecule.

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ˣ To whom inquiries should be directed.

Identification of Desmethylcyproheptadine-10,11-epoxide and Other Cyproheptadine Metabolites Isolated from Rat Urine

A. FRIGERIO ˣ, N. SOSSI, G. BELVEDERE, C. PANTAROTTO, and S. GARATTINI

Abstract □ Desmethylcyproheptadine-10,11-epoxide, cyproheptadine-10,11-epoxide, and desmethylcyproheptadine were identified in rat urine collected after administration of 40 mg/kg ip of cyproheptadine. Mass spectrometric characterization confirmed the structure of these metabolites.

Keyphrases □ Cyproheptadine—isolation of metabolites from rat urine, identification of desmethylcyproheptadine-10,11-epoxide, metabolism pathways suggested □ Desmethylcyproheptadine-10,11-epoxide—identification as a metabolite of cyproheptadine in rat urine, isolation of other metabolites □ Metabolism—cyproheptadine, identification of desmethylcyproheptadine-10,11-epoxide and other metabolites, rat urine

In previous studies it was established that carbamazepine (I) can be transformed in humans and rats into an epoxide in the 10,11-double bond (II) (Scheme I) (1). This finding suggested that other chemicals with similar structures could be metabolized to form an epoxide.

As a part of a systematic investigation, this paper describes the identification of some metabolites of

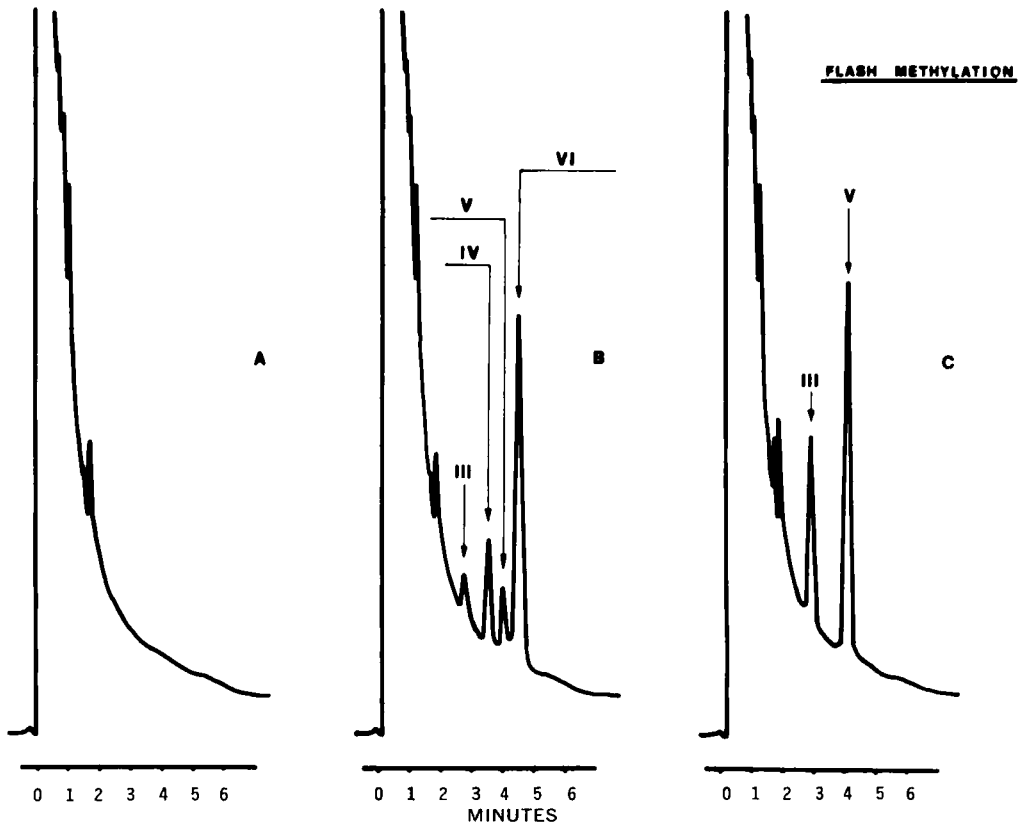


Figure 1—*GLC chromatograms. Key: A, urine blank; B, urine of rats treated with cyproheptadine (III), 40 mg/kg ip; and C, urine of treated rats after flash methylation using trimethylanilinium hydroxide. Retention times were: III, 2.8 min; IV, 3.5 min; V, 4.0 min; and VI, 4.5 min.*

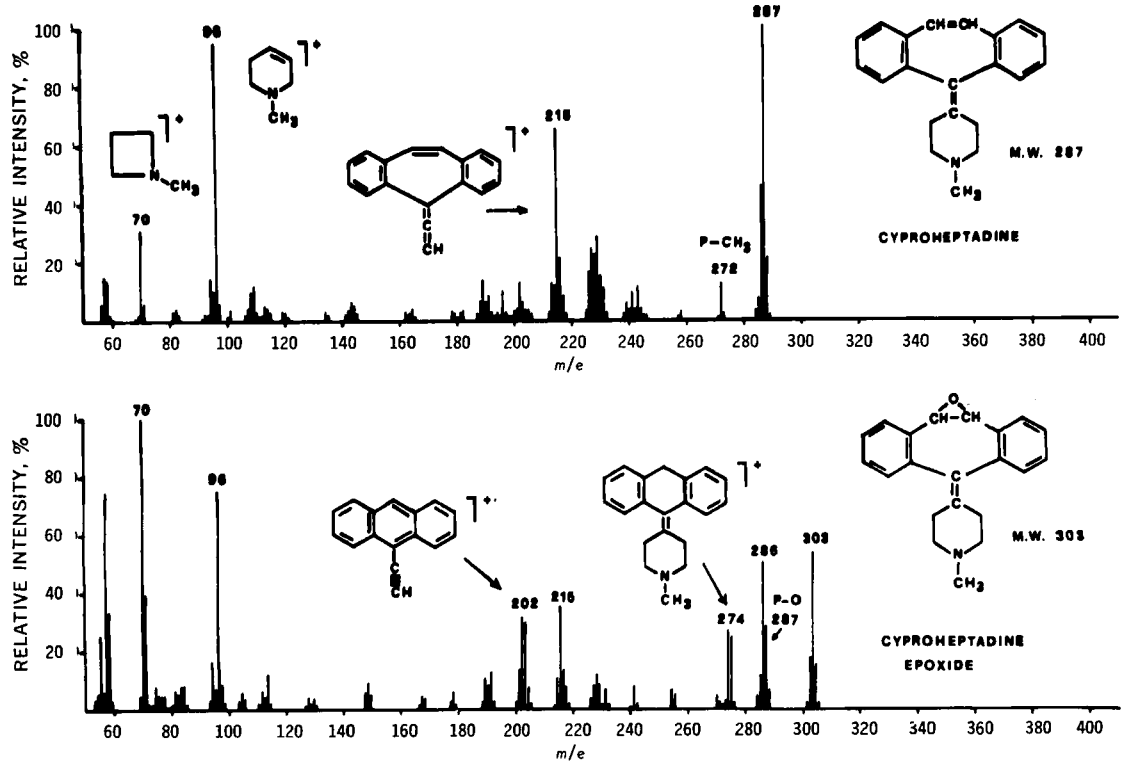
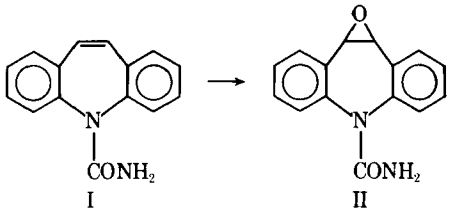


Figure 2—*Mass spectrum of authentic III and V. Similar mass spectra were obtained by introducing the corresponding eluted TLC spots by a direct inlet system or by a GLC procedure.*



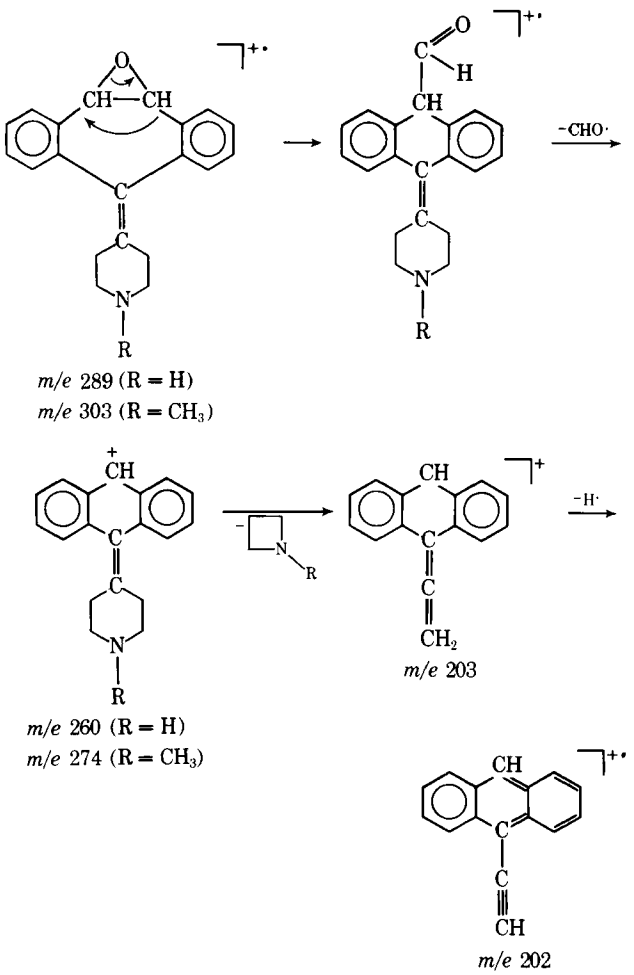
Scheme I—Epoxide formation from carbamazepine

cyproheptadine (III), 4-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-methylpiperidine including the product of desmethylation and the epoxides of both cyproheptadine and desmethylcyproheptadine (IV).

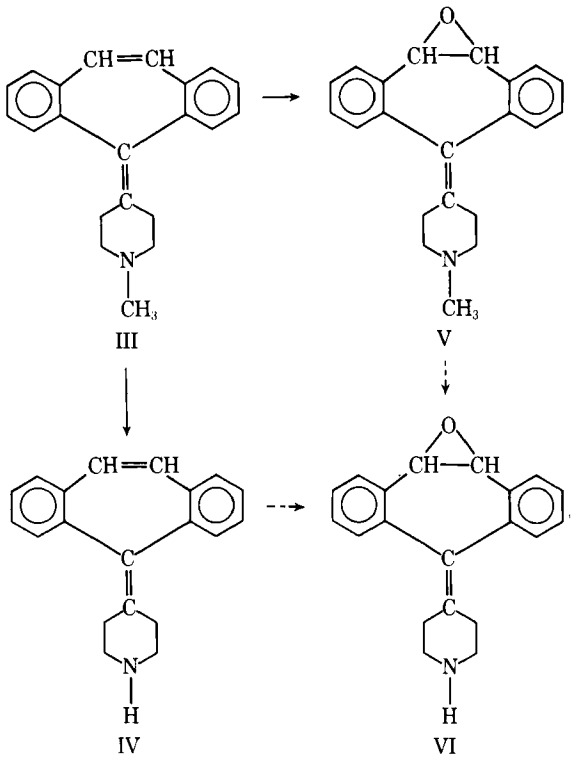
Cyproheptadine is a drug with antihistaminic and antiserotonin activities, and it is utilized for the treatment of allergic disorders (2) as well as for increasing the appetite of children and the elderly (3).

EXPERIMENTAL

Biological Samples—Two male Sprague–Dawley rats (200 g) were injected with 40 mg/kg ip of cyproheptadine[1] suspended in a 0.3% solution of hydroxypropyl cellulose (0.5 ml). Urine was collected at various times for 48 hr following drug administration and was kept frozen until analyzed. The drug, added to untreated rat urine and analyzed after 48 hr, did not show formation of any degradation products.



Scheme II—Suggested fragmentation pathway of cyproheptadine-10,11-epoxide and desmethylcyproheptadine-10,11-epoxide



Scheme III—Metabolic pathway of cyproheptadine: cyproheptadine (III), desmethylcyproheptadine (IV), cyproheptadine-10,11-epoxide (V), and desmethylcyproheptadine-10,11-epoxide (VI)

Urine samples (10–20 ml) were adjusted to pH 9 with 1 *N* NaOH and extracted with methylene chloride (20–40 ml). The organic phase was concentrated to dryness in a water bath at 65° under a gentle stream of nitrogen. This material was then used for TLC, GLC, and mass spectroscopic analyses.

General Procedures—Thin-layer chromatograms were prepared on 5 × 20-cm glass plates precoated with silica gel F-254[2] and developed at room temperature in carbon tetrachloride–methanol–diethylamine (85:10:5 v/v). Cyproheptadine and the possible metabolites were visualized under UV light at 254 nm.

GLC determinations were performed on a gas chromatograph[3] equipped with a flame-ionization detector. The chromatographic column was glass tubing (1 m long and 4 mm i.d.) packed with 100–120-mesh Gas Chrom Q and coated with 3% OV-17[4]. The operating conditions were: injection port temperature, 285°; oven temperature, 250°; detector temperature, 290°; nitrogen (carrier gas) flow rate, 50 ml/min; hydrogen flow rate, 20 ml/min; and air flow rate, 300 ml/min.

A gas chromatograph–mass spectrometer–computer system[5] was used at the following conditions: energy of the ionization beam, 70 ev; ion source temperature, 100°; and ionization current, 200 μamp. Sample introduction was carried out either by a direct inlet system at a probe temperature of 120° or by a GLC procedure utilizing a glass column (1 m long and 4 mm i.d.) packed with 3% OV-17 on 100–120-mesh Gas Chrom Q at the following conditions: injector temperature, 270°; oven temperature, 240°; and helium (carrier gas) flow rate, 30 ml/min.

Synthesis of Cyproheptadine-10,11-epoxide (V)—Cyproheptadine, 287 mg, was dissolved in 5 ml of acetone, and a mixture of 1 ml of acetonitrile and 2 ml of a 30% solution of hydrogen peroxide was added five times, keeping the pH constant at 9–10 with 1 *N* NaOH until oxygen was developed from the solution. After 48 hr at room temperature, the reaction mixture (20 ml) was extracted

[1] Donated by Merck Sharp & Dohme, Pavia, Italy.

[2] Merck.
[3] Carlo Erba Fractovap G1.
[4] Applied Science Laboratories.
[5] Finnigan quadrupole model 3100 and computer model 6000.

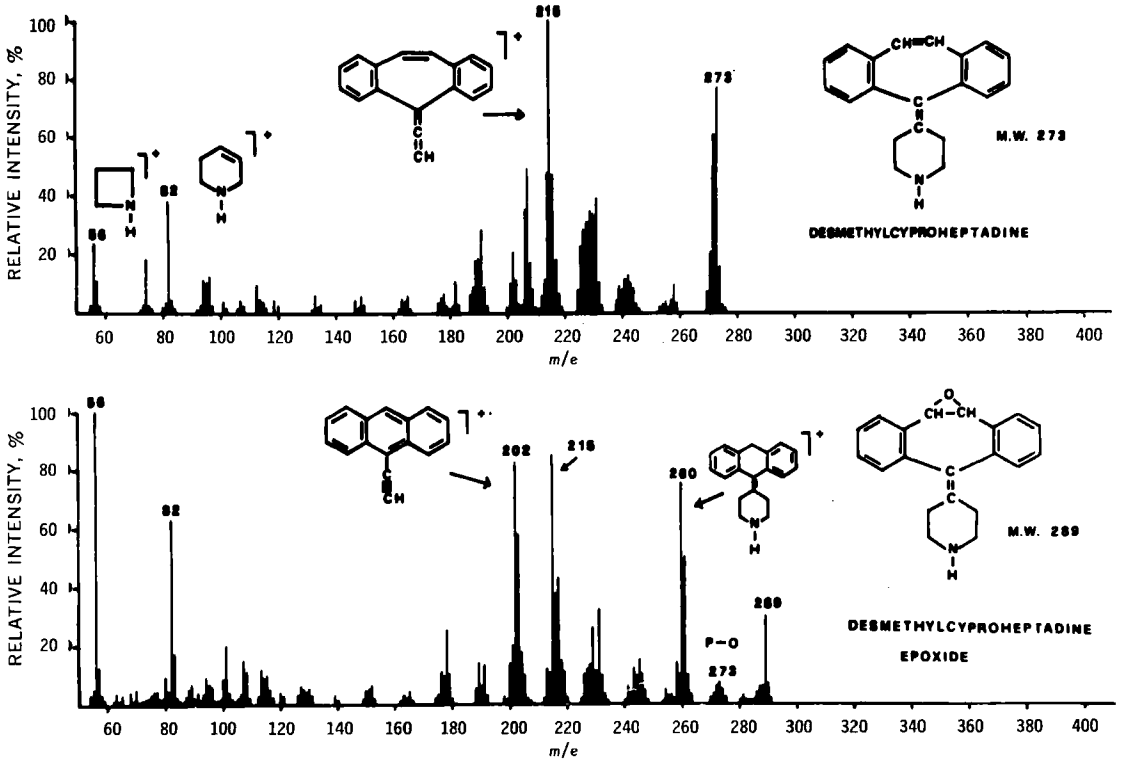


Figure 3—*Mass spectrum of IV and VI obtained by introducing the eluted TLC spots by a direct inlet system or by a GLC procedure.*

twice with an equal volume of methylene chloride, dried over sodium sulfate, and evaporated.

To reduce the *N*-oxide obtained from V, the solid was dissolved with 10 ml of 0.3 *N* $Na_2S_2O_4 \cdot 7H_2O$ and 5 ml of acetone. This solution was stored at room temperature for 15 hr. Then the solution was extracted twice with 15 ml of methylene chloride, and the organic layer was washed with 15 ml of 5% NH_4Cl. The organic phase was purified on a silica gel column (60 × 2 cm) eluted with cyclohexane–chloroform–diethylamine (70:20:10 v/v). The fractions collected (2 ml) were examined by GLC, and those containing the epoxide (retention time of 4.0 min) were pooled and evaporated.

The yield of epoxide, crystallized from methyl ethyl ketone, was 32%, mp 158–160°. The compound showed R_f 0.54 in the TLC system; NMR[6] ($CDCl_3$) showed: δ 4.22 (s, 2H,—HCO—epoxide), 2.21 (narrow s, 3H, N—CH_3), and 7.1–7.5 (m, 8H, ArH).

RESULTS AND DISCUSSION

The methylene chloride extract obtained from 20 ml of rat urine was concentrated to dryness, redissolved in 100 μl of methanol, and spotted on a TLC plate; carbon tetrachloride–methanol–diethylamine (85:10:5 v/v) was used as a solvent. The extracts of urine, obtained from rats treated with III (40 mg/kg ip), contained, in contrast to the extract of normal urine, four spots with R_f 0.60, 0.54, 0.47, and 0.39 when examined under UV light. The spot at R_f 0.60 corresponded to unchanged III, while the spot at R_f 0.47 corresponded to IV, a recently identified metabolite (4).

The GLC analyses showed that the methylene chloride extract of urines of cyproheptadine-treated rats, in contrast to controls, gave rise to four peaks (Fig. 1). The first two peaks corresponded to III and IV, respectively.

Further analyses were carried out by utilizing a GLC–mass spectroscopic procedure. The spot at R_f 0.60 eluted with methanol and introduced in the mass spectrometer with the direct inlet system and the substance yielding the first GLC peak gave a mass spectrum indistinguishable from the one obtained with authentic III (Fig. 2). The mass spectrum showed the molecular ion at *m/e* 287, corresponding to the base peak, and three main peaks at *m/e* 215, 96 (*N*-methyltetrahydropyridinil ion), and 70 (*N*-methylazetidinium ion), these two latter arising from the piperidine ring.

The mass spectrum of the eluate from the spot at R_f 0.54 and from the third peak obtained in GLC exhibited a molecular ion at *m/e* 303 (Fig. 2), suggesting, in respect to III, the gain of an oxygen atom as a result of the biotransformation. The fragmentation pathway suggests that the oxygen atom might be located at carbons 10 and 11 to form the epoxide derivative of III, as indicated in Scheme II. This scheme shows the possible fragmentation pathway of this molecule. The hypothesized structure was then confirmed by comparison with the mass spectrum of the synthetic compound.

The spot at R_f 0.47 and the second peak in GLC gave a mass spectrum corresponding to IV (Fig. 3). The mass spectrum of this metabolite showed a molecular ion at *m/e* 273 and other prominent peaks at *m/e* 215, 82, and 56; these latter two are due to a tetrahydropyridinil ion and an azetidinium ion, respectively.

The spot at R_f 0.39, as well as the fourth peak in GLC, was a substance with a molecular weight of 289 (Fig. 3). The fragmentation pathway is similar to V (Scheme II), the only differences being the peak at *m/e* 260 and the ions arising from the fragmentation of the piperidine ring (*m/e* 82 and 56). These findings suggest the introduction of an oxygen atom at carbons 10 and 11 in the structure of IV.

An indirect confirmation of this hypothesis was obtained by utilizing a flash methylation procedure (5). One microliter of the urine extract obtained from rats treated with III was injected into the gas chromatograph together with 1 μl of trimethylanilinium hydroxide. As shown in Fig. 1C, the peaks considered to be IV and desmethylcyproheptadine epoxide (VI) shifted to the peaks corresponding to the respective methylated compounds, III and V. The interpretation of the observed shift was then also confirmed by GLC–mass spectroscopic analysis.

The results obtained in rats suggest, but do not prove, that Scheme III is a possible metabolic pathway for III. The compound may undergo *N*-demethylation or epoxidation at carbons 10 and 11. The two metabolites may then be respectively epoxidated or *N*-demethylated to form the main metabolite VI.

Previous studies (6, 7) put forward the hypothesis that oxygenated metabolites of III could be responsible for the pancreatic toxicity induced in rats by high doses of the drug.

[6] Varian A60.

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[x] To whom inquiries should be directed.

Surface Activity of Prostaglandins E_2, $F_{2\alpha}$, A_1, and B_1 in Presence of Insoluble Monomolecular Films

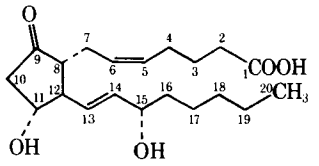
BERNARD SIMS [x] and SHELLY L. HOLDER [*]

Abstract □ Surface activities of four naturally occurring prostaglandins, PGE_2, $PGF_{2\alpha}$, PGA_1, and PGB_1, were investigated by examining the π–A compression isotherms obtained for insoluble monomolecular films of stearic acid spread on subphases containing the prostaglandins. All four compounds were observed to bring about increased instability of stearic acid monolayers, as evidenced by reductions in both transition and collapse surface pressures. Prostaglandin E_2 did not readily penetrate the monolayer but appeared capable of associating with the polar groups of the film, bringing about monolayer instability. PGB_1 exhibited a disruptive effect upon monolayer structure, causing some expansion of the π–A isotherm and increased instability. Prostaglandin $F_{2\alpha}$ penetrated into the stearic acid film, giving rise to increased surface pressure development in the initial regions of the π–A curve, resulting in a significantly expanded isotherm. This same effect, in the case of $PGF_{2\alpha}$, was observed using cholesterol and distearoyl phosphatidic acid monolayers. PGA_1 also appeared to penetrate the stearic acid monolayer, forming a mixed monolayer system and causing considerable rearrangement in monolayer structure as evidenced by an extended plateau region occurring in the π–A plot. The order of surface activities observed for the prostaglandins was $PGA_1 > PGB_1 > PGF_{2\alpha} > PGE_2$. The relative importance of polar functions, hydrophobic interactions, and hydrogen bonding are discussed with respect to the observed effects on monolayer stability. Some biological implications of the data are presented.

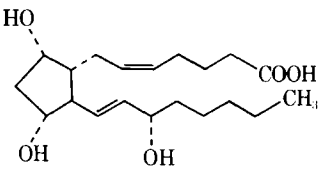
Keyphrases □ Prostaglandins E_2, $F_{2\alpha}$, A_1, and B_1—surface activity in presence of insoluble monomolecular films, π–A isotherms, biological implications □ Surface activity—four prostaglandins in presence of insoluble monomolecular films, π–A isotherms □ Films, insoluble monomolecular—surface activity of prostaglandins □ Monomolecular films, insoluble—surface activity of four prostaglandins

In recent years, great research interest has been directed toward the elucidation of the physical–chemical properties and pharmacological and physiological actions of a new class of hormones, the prostaglandins. Numerous review articles and publications have described their occurrence, synthesis, metabolism, and possible roles in biological processes (1–3). More recently, a journal has appeared devoted exclusively to prostaglandin research.

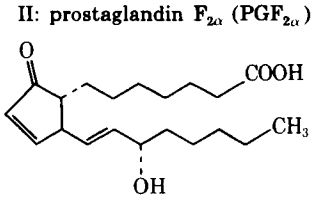
Surprisingly few investigations have concerned their surface activity (4, 5), especially since they have been implicated in various membrane-related functions, *e.g.*, vascular permeability changes (6), vascular resistance (7), intestinal transport of ions (8), binding of calcium to mitochondrial membrane (9), platelet aggregation (10), the inflammatory process (11), and smooth muscle stimulation (12). This study was undertaken to provide insight into the surface activity of four naturally occurring prostaglandins in the presence of simple model membrane systems.



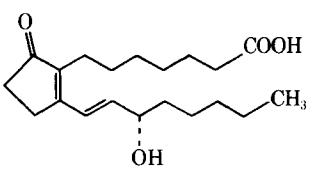
I: prostaglandin E_2 (PGE_2)



II: prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)



III: prostaglandin A_1 (PGA_1)



IV: prostaglandin B_1 (PGB_1)