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Keyphrases

Absorption rate, intrinsic—drugs
Drug absorption, distribution, elimination—models
Body considered—one, two compartments
Compartments, two *vs.* one—drug absorption
Kinetic equations—drug absorption
Acetylsalicylic acid salt—test compound
Griseofulvin—test compound

Effect of Topical Vehicle Composition on the *In Vitro* Release of Fluocinolone Acetonide and its Acetate Ester

By B. J. POULSEN, E. YOUNG, V. COQUILLA, and M. KATZ

A model was developed to test certain concepts regarding the *in vitro* release of steroids from topical vehicles. The steroids studied were fluocinolone acetonide and fluocinolone acetonide acetate. The vehicles selected for the study were propylene glycol–water mixtures gelled with Carbopol 934. Isopropyl myristate was used as the receptor phase for the diffusing steroids. Assay of the quantity of the steroids in the receptor phase was simplified by the use of radioactive compounds. The study indicated that the important factors influencing the release of steroid into the receptor phase were the solubility in the vehicle and the partition coefficient of the steroid between the vehicle and the receptor phase. Maximum release was achieved by altering the propylene glycol content of the vehicles.

OCCLUSIVE DRESSINGS have been successfully used to extend the therapeutic effectiveness of topical corticosteroids (1–3). This enhanced activity has been attributed to improved penetration of the skin by the drug (1). This indicates that, in certain instances, the development of formulations that increase drug penetration would be desirable.

There are two general approaches to the problem. One is to include in the vehicle agents which affect the barrier function of the epidermis so as to promote penetration of the therapeutic compound (4–7). The other approach is to alter the physical characteristics of the vehicle and thus affect the diffusion of the drug from the vehicle into the skin. As a preliminary to evaluating this latter effect of vehicles on skin penetration, the *in vitro* release of steroids from model vehicles was studied.

Higuchi (8) suggested the following equivalent relationships could be used to approximate the penetration of the barrier phase of the skin by a drug dissolved in a topical vehicle.

$$\frac{dQ}{dt} = (P.C.)C_v\frac{DA}{L} \qquad \text{(Eq. 1)}$$

$$\frac{dQ}{dt} = \frac{a_v}{\gamma_s}\frac{DA}{L} \qquad \text{(Eq. 2)}$$

The terms in these equations are defined as follows:

dQ/dt = steady rate of penetration
$(P.C.)$ = the effective partition coefficient of drug between skin barrier and vehicle
C_v = concentration of drug in the vehicle
D = the effective average diffusivity of the drug in the skin barrier
A = cross-sectional area of the application site
L = effective thickness of the skin barrier
a_v = thermodynamic activity of the drug in the vehicle

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γ_s = effective activity coefficient of the drug in the skin barrier

If Eqs. 1 and 2 are considered with the objective of altering the penetration of a topically applied drug, it becomes apparent that only certain terms are readily susceptible to manipulation by the formulator. These are the partition coefficient (*P.C.*) and drug concentration (C_v) terms of Eq. 1 and, equivalently, the activity term (a_v) of Eq. 2.

Relationships between partition coefficients of various compounds and skin penetration have been reported (9–13). For our purposes, the partition coefficient can be defined as

$$P.C. = \frac{\gamma_v}{\gamma_s} \qquad \text{(Eq. 3)}$$

where γ_v and γ_s are the activity coefficients of the drug in the vehicle and skin, respectively. While it may be necessary to regard the activity coefficient of the drug in the skin barrier phase as an unknown and unalterable value, γ_v can be modified simply by altering the composition of the vehicle. Higuchi (8) has reported, for instance, that the activity coefficient of Sarin is approximately 1,500 times greater in perfluorotributylamine than in *m*-cresol. Katz and Shaikh (9) have previously reported the correlation of solubility and partition coefficient to topical corticosteroid therapeutic activity as measured by the Stoughton-McKenzie vasoconstrictor test.

It is evident that increasing the thermodynamic activity of a topically applied drug by altering vehicle composition might be expected to enhance its rate of penetration. It is, however, also necessary to consider what can be accomplished with a specific drug relative to the concentrations actually required in therapy. In practical terms, the saturation solubility in the vehicle places an upper limit on the activity that can be achieved for the penetrant (8). In this study, a simple model system was devised to test the effect of vehicle composition on the release of therapeutic concentrations of two topically active corticosteroids.

EXPERIMENTAL

Materials—Carbopol 934,[1] diisopropanolamine,[2] isopropyl myristate,[3] propylene glycol,[4] toluene (scintillation grade),[5] PPO,[6] POPOP,[6] fluocinolone acetonide,[7] and fluocinolone acetonide acetate[8] were used as received. In the release experiments, ^{14}C-labeled (acetonide label) fluocinolone acetonide and fluocinolone acetonide acetate were used as provided by the Institute of Steroid Chemistry, Syntex Research, Palo Alto, California.

Preparation of Vehicles—The propylene glycol and propylene glycol–water mixtures used as the donor phase were gelled by the addition of 1% w/w Carbopol 934 resin. The Carbopol was neutralized by the addition of an equal weight of diisopropanolamine. The steroids were incorporated into the vehicles by dissolving the steroid in the propylene glycol portion of the vehicle. The Carbopol resin was dispersed in water or a portion of the propylene glycol with the aid of an Eppenbach Homo-Mixer and added to the steroid solution. Diisopropanolamine was then added to gel the mixture. The final pH of the preparations ranged from 6–7. Freshly prepared formulations were used in the release studies.

Since viscosity of a preparation could be a factor affecting release, viscosity determinations were made on gels containing from 1 to 100% propylene glycol. The measurements were made with a Brookfield model RVT viscometer using the Helipath stand. Determinations made at room temperature, using the TC spindle at 5 r.p.m., indicated that a slight decrease in viscosity occurred as the propylene glycol content of the gels was increased. This decrease amounted to approximately a 20% reduction in viscosity over the propylene glycol concentration range from 1 to 100%.

Determination of Release Rates—The containers used to support the gels in this study were Petri dishes 100 mm. in diameter and 5 mm. deep. The dish was completely filled with the vehicle and the excess removed with the edge of a spatula to produce an even, uniform surface of constant dimensions. The weight of vehicle required to fill the dish was approximately 30 Gm. The dish containing the vehicle was placed in the bottom of a 1,000-ml. beaker, and the beaker was partially immersed in a large water bath at 37°. Two-hundred milliliters of isopropyl myristate previously equilibrated to 37° was carefully layered over the vehicle to begin the experiment. A large stirring blade of a width approximately equal to the diameter of the Petri dish was employed to produce agitation. The bottom edge of the stirring blade was positioned just above the surface of the gel. Six experiments could be run simultaneously in the same water bath by the use of a multiple-blade Phipps-Bird laboratory stirrer. It was determined experimentally that the release rates were sensitive to the rate of stirring up to approximately 30 r.p.m. Accordingly, the stirring rate was maintained at 40 r.p.m. Samples of isopropyl myristate were withdrawn for assay at intervals over a 24-hr. period.

Solubility Determinations—The solubilities of fluocinolone acetonide and fluocinolone acetonide acetate in propylene glycol–water mixtures were determined by two methods. In the first, excess steroid was rotated in sealed 10-ml. ampuls in a constant-temperature bath at 25°. Samples were withdrawn for chemical analysis after 2 weeks. In the second method, excess steroid was heated to 80°,

[1] Supplied by B. F. Goodrich Co., Cleveland, Ohio.
[2] Union Carbide Chemicals Co., New York, N. Y.
[3] Deltyl-Extra by Givaudan Co., Clifton, N. J.
[4] Union Carbide Chemicals Co., New York, N. Y.
[5] Toluene (scintillation and fluorometric grade) from Matheson, Coleman & Bell, Div. Matheson Co., Inc., Norwood, Ohio.
[6] Arapahoe Chemicals, Div. Syntex Corp., Boulder, Colorado.
[7] 6α,9α-Difluoro-16α hydroxyprednisolone 16,17-acetonide (Synalar), Syntex Laboratories, Inc., Palo Alto, California.
[8] 6α,9α-Difluoro-16α hydroxyprednisolone 16,17-acetonide 21-acetate, Syntex Laboratories, Inc., Palo Alto, California.

with rapid stirring for a few minutes, and the resulting mixture sealed into 10-ml. ampuls and rotated in the constant-temperature bath at 25° for 24 hr. prior to analysis. Equivalent results were obtained by the two methods.

The samples were assayed by withdrawing 5 ml. of solution, filtering through a Millipore filter (type GS, mean pore diameter 0.22 ± 0.02 μ), diluting with water, extracting the steroid with chloroform, evaporating the chloroform extract to dryness, taking up the steroid with ethanol, and assaying a suitable dilution of the alcoholic solution by the blue tetrazolium colorimetric assay.

Partition Coefficient Determinations—One-tenth milligram of steroid per ml. was dissolved in isopropyl myristate. Twenty-five milliliters of this solution was added to an equal volume of the various propylene glycol–water mixtures and equilibrated at 37°. After equilibrium, the phases were separated and assayed individually for steroid content. Partition coefficients were calculated as the ratio of the steroid concentration in the isopropyl myristate to that in the glycol–water phase.

Radiochemical Assays—The specific activities of the radiochemicals used in the study were 0.20 μc./mg. for fluocinolone acetonide-^{14}C and 0.10 μc./mg. for fluocinolone acetonide acetate-^{14}C. Samples of the isopropyl myristate receptor phase were assayed for steroid by counting in a Packard Tri-Carb liquid scintillation counter. Two-milliliter samples were mixed directly with 15 ml. of scintillation fluid consisting of 5 Gm. of PPO (2,5-diphenyloxazol), and 0.1 Gm. of POPOP (1,4-bis-2-[4-methyl-5-phenyloxazolyl]benzene) diluted to 1 L. with scintillation grade toluene. Internal standardizations were used to evaluate quenching of the samples. Ordinarily, quenching was found to be negligible and no quenching correction was required. Since steroids of low specific activity were used in the study, samples were counted for a total of 10 min. Total steroid release was calculated from the specific activities of the isotopes.

Confirmation that the radioactive assay measured only intact steroid was accomplished as follows: Samples of isopropyl myristate were withdrawn following release experiments of 24-hr. duration. The steroid was extracted from the isopropyl myristate and spotted from chloroform solution on silica gel TLC plates. The plates were developed in chloroform–butyl acetate–acetone (2:2:1) and dried. The plates were then scanned for radioactivity in a Vanguard model 880 dual-channel autoscanner equipped for thin-layer plates. It was found that essentially all the radioactivity was concentrated in a plate region corresponding to the correct R_f value for the steroid in question.

RESULTS

The solubilities of fluocinolone acetonide and the 21-acetate ester in propylene glycol–water mixtures at 25° are shown in Fig. 1. Partition coefficients for the two steroids between isopropyl myristate and various propylene glycol–water mixtures were determined at 37° and are shown in Fig. 2. Since the composition of the isopropyl myristate remained essentially unchanged during the release experiments, the partition coefficients are a reasonable approximation of the relative activity coefficients for the drug in the vehicles.

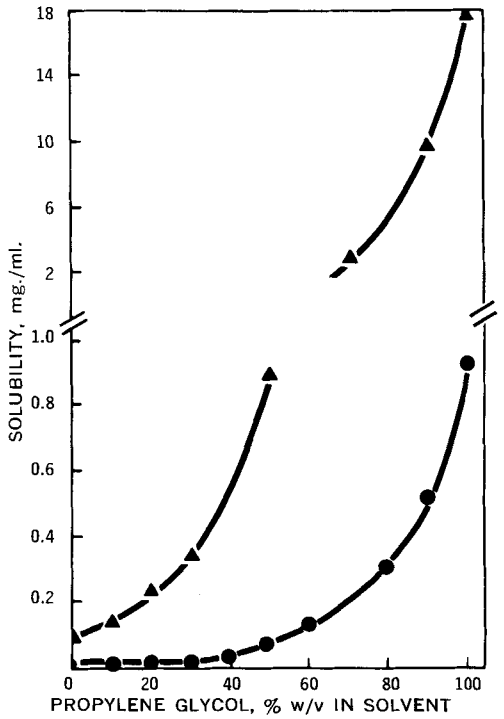

Fig. 1—Solubility of fluocinolone acetonide and its acetate ester in propylene glycol–water mixtures at 25°. Key: ▲, fluocinolone acetonide; ●, fluocinolone acetonide acetate.

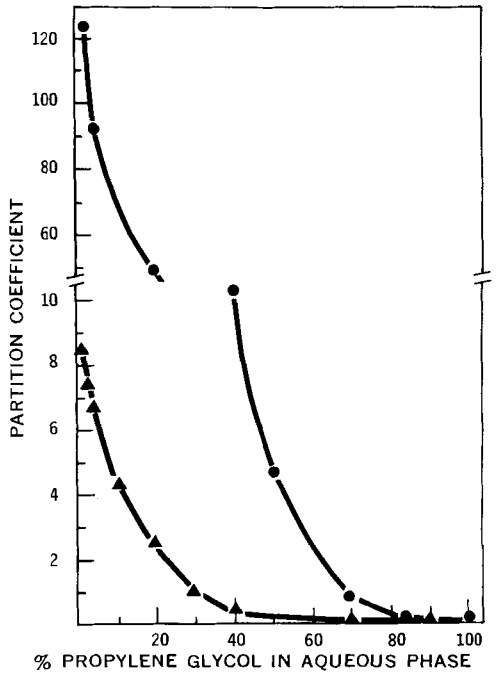

Fig. 2—Partition coefficients for fluocinolone acetonide and its acetate ester between propylene glycol–water mixtures and isopropyl myristate at 37°. Key: ▲, fluocinolone acetonide; ●, fluocinolone acetonide acetate.

Figure 3 is a plot of the total release of fluocinolone acetonide after 2, 6, and 12 hr. at 37° from gels containing various concentrations of propylene glycol. Optimal release for 0.025% fluocinolone acetonide preparations occurred with gels containing approximately 20% propylene glycol. As seen in Fig. 1 this concentration of propylene glycol is only slightly less than the minimum amount estimated to be required to completely solubilize 0.025% fluocinolone acetonide in the vehicle. At lower concentrations of propylene glycol, precipitated steroid appeared in the gel. Although reduced release rates were observed with gels containing mostly undissolved steroid, the poorest release was actually obtained with gels containing very high concentrations of propylene glycol.

Somewhat different results were found for the less soluble fluocinolone acetonide acetate (Fig. 4). The solubility data obtained for the acetate ester (Fig. 1) indicated that about 75% propylene glycol would be required to solubilize this compound at a concentration of 0.025%. Maximum release at 37° occurred from vehicles containing approximately 70% propylene glycol. In contrast to fluocinolone acetonide, the poorest release occurred from gels containing low propylene glycol concentrations in which a relatively large proportion of the steroid was present as undissolved material.

Figure 5 compares the release of the two steroids from several of the vehicles tested over a 24-hr. period. Figure 6 gives a comparison of percent steroid released after 6 hr. from vehicles containing 0.01, 0.025, and 0.1% fluocinolone acetonide.

These data are reasonably consistent with the observation that maximum release for various steroid concentrations in the vehicles studied occurred from gels containing the minimal amount of propylene glycol required to dissolve the compound. Figure 6 also shows the result of the increase in the activity

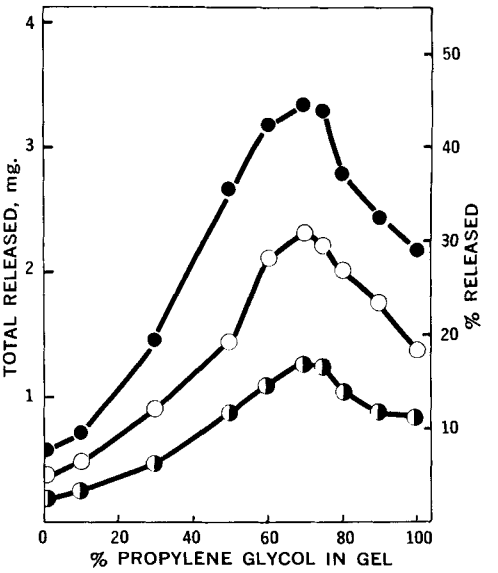

Fig. 4—Effect of propylene glycol concentration on the release of 0.025% fluocinolone acetonide acetate from propylene glycol–water gels at 37°. Key: release after— ◑, 2 hr.; ○, 6 hr.; ●, 12 hr.

coefficient that could be achieved at the lower (0.01 and 0.025%) fluocinolone acetonide concentrations. While total release was greater for the higher concentrations, the percent released over a 6-hr. time period was much greater for the 0.01% steroid concentration.

DISCUSSION

A number of studies describing the release of drugs from topical vehicles are reported in the literature. When the vehicle and receptor phase are mutually

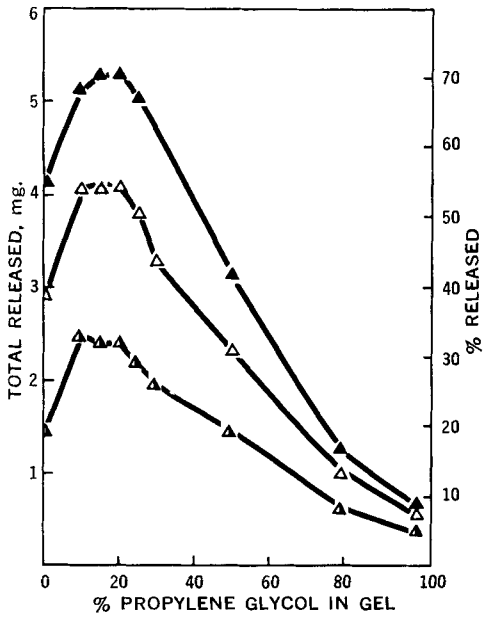

Fig. 3—Effect of propylene glycol concentration on the release of 0.025% fluocinolone acetonide from propylene glycol–water gels at 37°. Key: release after— △, 2 hr.; △, 6 hr.; ▲, 12 hr.

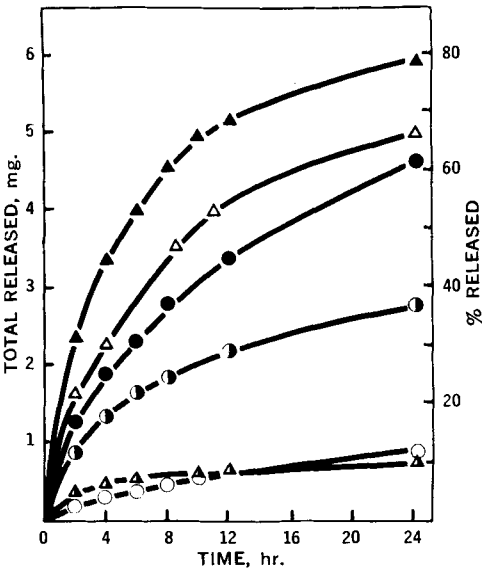

Fig. 5—Effect of propylene glycol concentration on the release of 0.025% steroid from Carbopol gels. Key: concentration of propylene glycol in fluocinolone acetonide gels—△, 1%; ▲, 20%; ◭, 100%; concentration in fluocinolone acetonide acetate gels—○, 1%; ●, 70%; ◑, 100%.

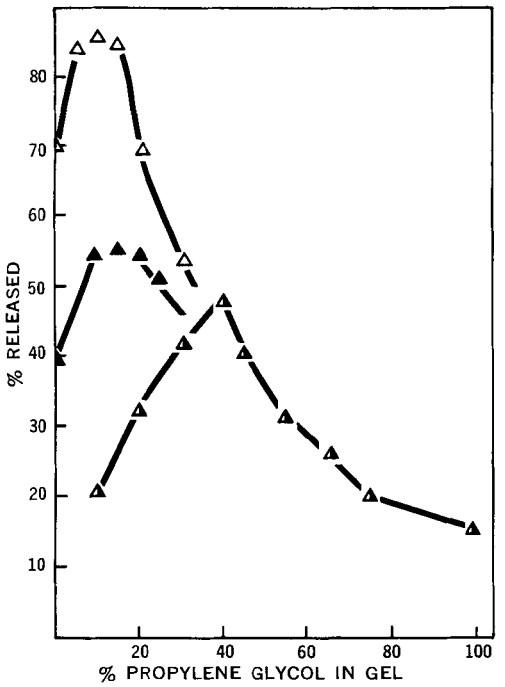

Fig. 6—Comparison of release of 0.01%, 0.025%, and 0.1% fluocinolone acetonide from propylene glycol–water gels after 6 hr. at 37°. Key: % steroid—△, 0.01%; ▲, 0.025%; ◭, 0.1%.

miscible, physical separation of the two phases is necessary. A dialysis membrane has frequently been used for this purpose (14–18). In this study, isopropyl myristate was employed instead of water as the receptor phase to more closely simulate skin lipids or skin itself. Since isopropyl myristate is immiscible with water and propylene glycol, it was not necessary to introduce an artificial membrane to separate it from the vehicles used in this study. The solubilities of fluocinolone acetonide (0.9 mg./ml.) and fluocinolone acetonide acetate (0.2 mg./ml.). at 37° were adequate for this lipid-like material to be used as the receptor phase.

Propylene glycol–water mixtures gelled with Carbopol 934 were selected as vehicles because steroid solubility could be readily altered by varying the concentration of propylene glycol. Solubility data for the two steroids in propylene glycol–water mixtures were determined at 25° (Fig. 1) and used to estimate the solubility in these same mixtures gelled with Carbopol. Microscopic examination of the vehicles indicated that addition of the gelling agent resulted in negligible changes in the solubility of the steroids in the glycol–water mixtures.

Partition coefficients were determined between glycol–water mixtures and isopropyl myristate (Fig. 2) and, as indicated by Eq. 3, were useful as an index of the relative activity coefficients for the steroids in these vehicles. This information was particularly valuable in estimating the potential effect of changes in the steroid concentrations in these vehicles on the release rate.

The possibility exists that molecular interactions between the steroids and the carboxyvinyl polymer gelling agent were a factor affecting the release rates. In addition, the solubility data for the steroids in glycol–water mixtures were obtained at room temperature (25°), whereas the release experiments were conducted at 37°. Consequently, steroid solubility in the gels under the conditions of the experiments was probably slightly greater than shown in Fig. 1. This may account in part for the observation that maximum release of 0.1% fluocinolone acetonide (Fig. 6) occurred from vehicles containing somewhat less propylene glycol than required to completely dissolve the steroid at room temperature. For these reasons, the solubility and partition coefficient data developed with the glycol–water mixtures were used only as an aid in interpreting the release data.

Maximum release for a given concentration of either fluocinolone acetonide or fluocinolone acetonide acetate was obtained from vehicles containing approximately the minimum amount of propylene glycol necessary to dissolve the steroid completely. Excess propylene glycol increased the affinity of the vehicle for the steroid and resulted in a decrease in steroid release. When insufficient propylene glycol was present to dissolve all the steroid, then diffusion into the receptor phase became dissolution rate limited and the release rate was reduced. As might be expected, the latter effect was much more pronounced with the less soluble compound, fluocinolone acetonide acetate.

The results obtained in this study clearly demonstrate the dependence of steroid release on the physical properties of both steroid and vehicle. For steroid concentrations of 0.025%, a vehicle containing 20% propylene glycol releases approximately 70% of available fluocinolone acetonide but only 15% of the acetate ester after 12 hr. Likewise, a vehicle containing 70% propylene glycol releases 45% of the acetate ester and only 25% of fluocinolone acetonide. Clearly, a vehicle that provides good release of one drug may be a poor vehicle for another closely related compound. The results obtained with different concentrations of the same steroid are of particular interest (Fig. 6). A much higher percent release over a given time interval could be obtained from 0.01% fluocinolone acetonide gels than from vehicles containing higher concentrations of steroid. In this case, higher partition coefficients were obtained with the lower steroid concentration while completely solubilizing the steroid. The obvious implication is that vehicle composition can be varied not only to improve release of different steroids but also specific concentrations of a given steroid.

Release studies have long been used as one criterion for judging the possible effect of vehicle on drug availability from topical formulations. There are obvious limitations of such methods, and conclusions must be drawn with great caution. Certainly the partition coefficient for a steroid between vehicle and isopropyl myristate will not duplicate that between vehicle and human skin. But, regardless of the actual value of the partition coefficient for a penetrant present on the surface of the skin, it must be subject to its environment. If that environment is a pharmaceutical vehicle, the composition of that vehicle should affect the passage of penetrant into an adjacent phase whether that phase is sebum or skin tissue. The degree to which this is actually true, and its clinical implications, must ultimately be established by correlation to *in vivo* studies using the same topical formulations.

SUMMARY

Isopropyl myristate was used as the receptor phase to evaluate the release of fluocinolone acetonide and its acetate ester from gelled propylene glycol–water vehicles. Steroid release was found to be a function of its (*a*) concentration, (*b*) solubility in the vehicles, and (*c*) partition coefficient between vehicles and receptor phase. Release was reduced if a vehicle contained cosolvent in excess of that required to dissolve the steroid. Release could be improved not only for the different steroids, but also for different concentrations of the same steroid, by use of the proper vehicle.

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Keyphrases

Vehicles, topical—steroid release
Propylene glycol–water–Carbopol 934—vehicles
Fluocinolone acetonide and its acetate–test compounds
Radioactive compounds—release rates
Liquid scintillation counting—analysis
Solubility—propylene glycol–water
Partition coefficient—isopropyl myristate–propylene glycol system

Euphorbia esula L. (*Euphorbiaceae*) I

Preliminary Phytochemical and Biological Evaluation

By N. R. FARNSWORTH*, H. WAGNER, L. HÖRHAMMER, H. P. HÖRHAMMER, and H. H. S. FONG

The aerial parts of *Euphorbia esula* were subjected to a fractionation scheme which resulted, after several chromatographic separations, in the isolation of β-sitosterol, 24-methylenecycloartanol, hexacosanol-(1), *n*-nonacosane, and *n*-hentriacontane. The presence of *n*-pentacosane, *n*-hexacosane, *n*-heptacosane, *n*-octacosane, *n*-triacontane, *n*-dotriacontane, and *n*-tritriacontane was demonstrated by means of gas chromatography. None of these compounds had previously been reported as isolated from *Euphorbia esula*. The presence of flavonoids and alkaloids was demonstrated, a finding that is contrary to literature reports. A defatted ethanol extract elicited no antimicrobial activity against several test organisms and was found to induce only weak central nervous system depression in mice. Seven tumor systems were not inhibited by the extract, and it was not cytotoxic in cell culture.

LIMITED phytochemical investigations on *Euphorbia esula* L. have shown only the presence of L-inositol (1), gallic acid (2), rubber (2), and a number of common amino acids (3). Conversely, phytochemical screening of this plant has indicated the absence of hemolytic saponins, flavonoids, alkaloids, and tannins (4). Peroxidase and polyphenoloxidase activity has been reported for rootbud extracts (3), and an inhibitor of indole-3-acetic acid has been shown to be present in whole plant extracts (5).

Folkloric usage for the treatment of cancer has been recorded for several species of *Euphorbia*

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