Table I—*In Vitro* Inhibition of Rat Lens Aldose Reductase by Various Substances

Inhibitor	Inhibition[a], %			
	10^{-4} *M*	10^{-5} *M*	10^{-6} *M*	10^{-7} *M*
Total crude flavonoid fraction	74	37	0	0
Flavocannabiside	40	10	0	0
Flavosativaside	35	0	0	0
Orientin	90	82	45	0
Quercitrin	100	95	88	55

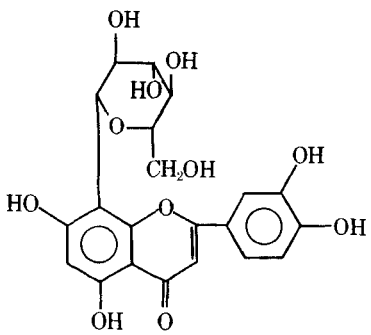
[a] Percentage of inhibition of the aldose reductase activity when compared with controls containing no inhibitors.

three isolated marijuana flavone C-glycosides as potential lens aldose reductase inhibitors.

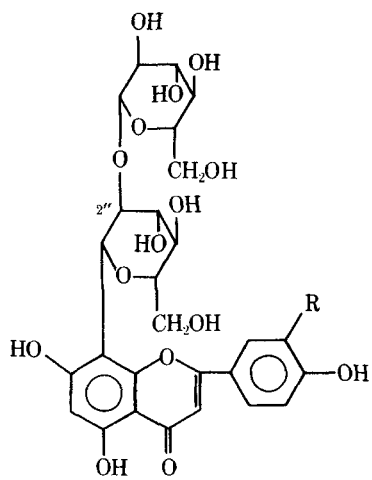
In the present study, I–III as well as the total crude flavonoid fraction from which they were isolated were each tested for inhibitory activity against a partially purified rat lens aldose reductase enzyme according to previously reported methods (3). Each substance was tested four to six times; the results shown in Table I represent mean values. The standard deviation of the results was less than 5%.

The C-diglycosylflavones II and III were relatively weak inhibitors. The C-monoglycosylflavone I proved to be 45% inhibitory at 10^{-6} *M* and compared favorably with the flavonol glycoside IV, which caused a 55% inhibition at 10^{-7} *M*.

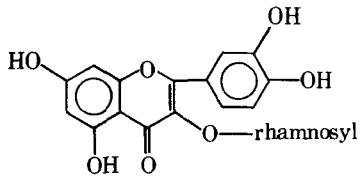
Work is underway to determine the inhibitory effect of I on aldose reductase in a rat lens organ culture assay (4),



I



II: R = OH
III: R = H



IV

and studies using additionally isolated marijuana flavonoids are anticipated[3].

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[3] *Added in proof:* The following recent reports describe further work dealing with diabetic cataracts and flavonoids: S. D. Varma and J. H. Kinoshita, *Biochem. Pharmacol.*, **25**, 2505 (1976); S. D. Varma, A. Mizuno, and J. H. Kinoshita, *Science*, **195**, 205 (1977).

Use of *In Vitro* Dissolution Data to Predict Plasma Drug Profiles

Keyphrases □ Plasma time profiles—predicted from *in vitro* dissolution data, validity evaluated □ Dissolution rates, *in vitro*—used to predict plasma time profiles, validity evaluated

To the Editor:

Recently, Vaughan and Leach (1) discussed a simple method for predicting plasma time profiles from *in vitro* dissolution data. Basically, their method is to relate the

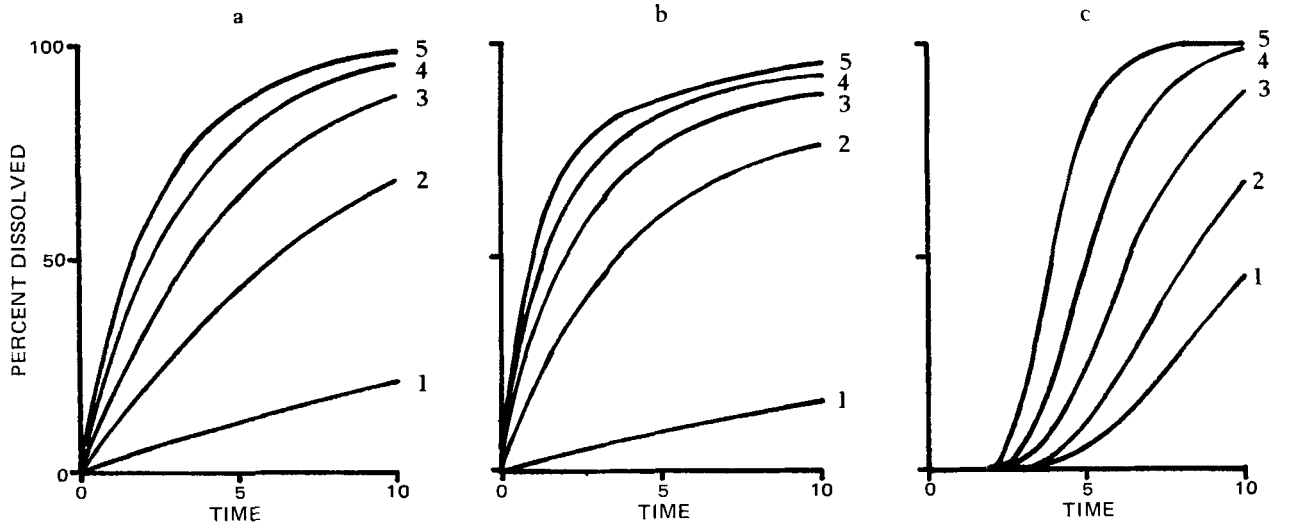


Figure 1—*Dissolution profiles from different formulations of a drug when dissolution is governed by linear (a), cube root (b), and log normal (c) dissolution laws. Parameters were adjusted so that the fastest dissolving formulation reached 90% dissolution at the same time for each law and the slowest dissolving formulation had reached 10% dissolution at this time.*

amount of drug dissolved at time t in an *in vitro* dissolution test, $dis_j(t)$, to the plasma drug concentration obtained following administration of an oral dose of the preparation, $Cp_j(t)$, *via:*

$$dis_j(t) = H(t)Cp_j(t) \qquad \text{(Eq. 1)}$$

where j refers to the jth formulation. Thus, if $H(t)$ is a specific function of t and is independent of the formulation, then the following relation exists between two different preparations, i and j:

$$\frac{dis_i(t)}{dis_j(t)} = \frac{Cp_i(t)}{Cp_j(t)} \qquad \text{(Eq. 2)}$$

Potentially, this method could be very useful in drug design or for predicting plasma profiles, avoiding many detailed pharmacokinetic analyses. However, $H(t)$ is not a formulation-independent function (even when dealing with linear pharmacokinetics as suggested by Vaughan and Leach), so the validity of Eq. 1 was investigated based on some simple pharmacokinetic models. Although it may be argued that these models are idealized, Eq. 1 was offered as a quite general relationship (although certain sensible restrictions were placed on its use by the authors which will be discussed later), and it must stand the test of abstracted models.

Table I—Calculated Values of $H(t)$ for Formulations with Drug Release Patterns Depicted in Fig. 1, Using Eq. 1, with $k_2 = 5$ and $k_3 = 0.5$

	Linear[a]					Cube Root					Log Normal				
Time	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1.0	1.528	1.523	1.516	1.509	1.503	1.528	1.515	1.493	1.477	1.466	3.565	4.421	4.774	5.239	5.833
2.0	1.693	1.699	1.708	1.718	1.728	1.694	1.709	1.747	1.783	1.818	2.060	2.192	2.227	2.260	2.291
3.0	2.003	2.041	2.090	2.143	2.199	2.009	2.087	2.253	2.393	2.512	1.724	1.729	1.722	1.708	1.684
4.0	2.376	2.471	2.600	2.740	2.888	2.390	2.582	2.972	3.293	3.560	1.650	1.630	1.621	1.611	1.602
5.0	2.795	2.983	3.247	3.543	3.867	2.820	3.190	3.942	4.561	5.080	1.691	1.702	1.719	1.755	1.830
6.0	3.250	3.576	4.056	4.617	5.251	3.292	3.920	5.216	6.308	7.240	1.805	1.908	1.989	2.134	2.401
7.0	3.736	4.256	5.060	6.049	7.215	3.800	4.778	6.859	8.669	10.250	1.979	2.260	2.468	2.833	3.478
8.0	4.249	5.027	6.302	7.960	10.010	4.341	5.774	8.935	11.795	14.367	2.213	2.803	3.247	4.027	5.363
9.0	4.785	5.897	7.833	10.513	14.010	4.911	6.914	11.511	15.850	19.875	2.514	3.618	4.484	6.019	8.559
10.0	5.341	6.873	9.716	13.929	19.758	5.507	8.203	14.648	20.995	27.076	2.892	4.836	6.444	9.314	13.900

[a] The numbers refer to the dissolution profiles in Fig. 1.

Table II—Calculated Values of $H(t)$ for Formulations with Drug Release Patterns Depicted in Fig. 1, Using Eq. 1, with $k_2 = 5$ and $k_3 = 0.1$

	Linear					Cube Root					Log Normal				
Time	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1.0	1.300	1.291	1.283	1.275	1.267	1.299	1.269	1.246	1.228	1.213	5.789	4.784	4.179	3.768	3.466
2.0	1.214	1.207	1.201	1.195	1.190	1.213	1.193	1.183	1.176	1.173	2.161	2.101	2.035	1.971	1.912
3.0	1.229	1.226	1.224	1.223	1.223	1.228	1.225	1.228	1.232	1.237	1.478	1.516	1.525	1.521	1.509
4.0	1.266	1.270	1.276	1.282	1.289	1.267	1.285	1.302	1.317	1.330	1.251	1.302	1.331	1.344	1.349
5.0	1.314	1.328	1.343	1.358	1.375	1.316	1.360	1.395	1.420	1.441	1.197	1.227	1.253	1.270	1.279
6.0	1.369	1.394	1.422	1.450	1.478	1.372	1.448	1.501	1.539	1.567	1.232	1.222	1.233	1.244	1.253
7.0	1.427	1.469	1.512	1.556	1.599	1.432	1.548	1.621	1.671	1.709	1.320	1.263	1.248	1.248	1.251
8.0	1.489	1.551	1.615	1.678	1.737	1.496	1.657	1.754	1.819	1.866	1.441	1.337	1.290	1.273	1.268
9.0	1.555	1.640	1.729	1.815	1.894	1.564	1.778	1.901	1.982	2.040	1.586	1.440	1.353	1.314	1.297
10.0	1.623	1.738	1.857	1.970	2.071	1.635	1.910	2.063	2.163	2.233	1.750	1.566	1.438	1.370	1.338

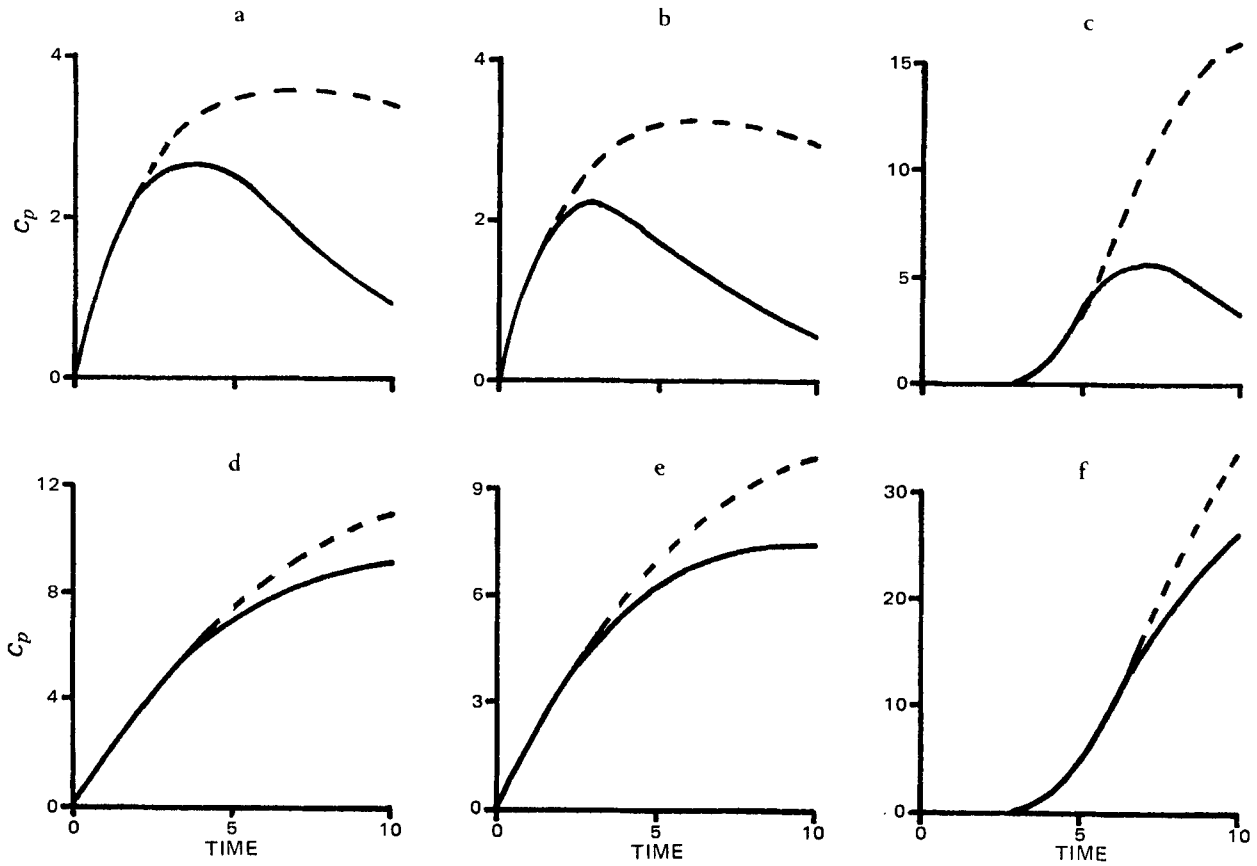
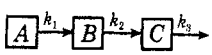


Figure 2—*Predicted (—) and correct (- - -) plasma profiles. Key: a, b, and c, linear, cube root, and log normal dissolution laws, respectively, with* $k_2 = 5$ *and* $k_3 = 0.5$*; and d, e, and f, linear, cube root, and log normal dissolution laws, respectively, with* $k_2 = 5$ *and* $k_3 = 0.1$.

One-Compartment Model—In the linear dissolution model shown in Scheme I, A, B, and C represent the



Scheme I

amounts of drug in the dosage form, in solution at the absorption site, and in the body, respectively; k_1, k_2, and k_3 are the corresponding first-order rate constants for the respective processes. The *in vitro* and *in vivo* dissolution profiles are assumed to be the same and are given by:

$$\text{dis}(t) = 100(1 - e^{-k_1 t}) \qquad \text{(Eq. 3)}$$

expressed as a percentage. In the same units, the time course of the drug in the body is:

$$Cp(t) = \frac{100k_1k_2[(k_2 - k_3)e^{-k_1 t} - (k_1 - k_3)e^{-k_2 t} + (k_1 - k_2)e^{-k_3 t}]}{(k_1 - k_2)(k_2 - k_3)(k_1 - k_3)} \qquad \text{(Eq. 4)}$$

such that:

$$H(t) = \frac{(k_1 - k_2)(k_2 - k_3)(k_1 - k_3)(1 - e^{-k_1 t})}{k_1k_2[(k_2 - k_3)e^{-k_1 t} - (k_1 - k_3)e^{-k_2 t} + (k_1 - k_2)e^{-k_3 t}]} \qquad \text{(Eq. 5)}$$

Differences in formulation are embodied in the parameter k_1; the larger k_1 is, the faster the drug dissolves.

It can be seen that $H(t)$ is a function of k_1 and is not formulation independent, as suggested in the introduction. It is instructive to look at several limiting forms of this function. In Case 1, $k_1 \gg k_2, k_3$. The drug dissolves so rapidly that it is effectively instantaneously available for absorption. By neglecting k_2 and k_3 with respect to k_1 and $e^{-k_1 t}$ with respect to $e^{-k_2 t}$ and $e^{-k_3 t}$, $H(t)$ becomes:

$$H(t) \sim \frac{(k_3 - k_2)}{k_2[e^{-k_2 t} - e^{-k_3 t}]} \qquad \text{(Eq. 6)}$$

Although $H(t)$ is now independent of k_1, it is useless for predictive purposes because it just relates a step function input to the plasma profile.

In Case 2, $k_2, k_3 \gg k_1$. Dissolution is rate limiting, and the limiting form of $H(t)$ approaches:

$$H(t) \sim \frac{k_3(1 - e^{-k_1 t})}{k_1 e^{-k_1 t}} \qquad \text{(Eq. 7)}$$

Here we find the most valid application of the method in that the plasma profile directly parallels the dissolution profile in the early phase ($k_1 t < 1$).

In Case 3, $k_1, k_2 \gg k_3$. The example of alprenolol discussed by Vaughan and Leach (1) approximately fits this category, and $H(t)$ reduces to $e^{k_3 t}$. Thus, as expected, after rapid dissolution and absorption, the plasma profile shows a simple exponential decline, reflecting elimination of drug from the body.

Simulations—The various limiting forms of $H(t)$ do not allow an assessment of how well or how badly this method works over an extended time scale. Therefore, it was necessary to resort to numerical integration. The studies were further extended to include two other kinds of dissolution,

cube root law (2) and log normal[1] (3). So that a meaningful comparison could be made between the three dissolution laws, it was arranged that $dis_j(t)$ for the fastest dissolving formulation reached 90% at the same time for each law and that the slowest dissolving formulation had reached 10% at this time. The rate constants k_2 and k_3 were fixed at 5 and 0.5 in one trial and at 5 and 0.1 in another. The time units are arbitrary.

The dissolution profiles for the three laws are shown in Fig. 1, and the values of $H(t)$ calculated using Eq. 1 are listed in Tables I and II. To obtain a better appreciation for the numbers, the plasma profile appropriate to the slowest dissolving formulation was calculated using the $H(t)$ appropriate to the fastest dissolving formulation and compared to the correct profile for each dissolution law. These values are displayed in Fig. 2.

Discussion—Although extensive parameter searches were not carried out, general trends revealed themselves. First, it is surprising how well the method works in these simulations at early times, *i.e.*, when dissolution is not complete. Vaughan and Leach (1) stressed this finding, and it is only reasonable that the method must break down when the drug in the fastest dissolving formulation has completely dissolved. As mentioned earlier, the method will be of most use when the dissolution rate limits input to the body. This condition was ensured in these simulations by having the drug pass rapidly through compartment B such that the ratio of $A/(A + B)$ was nearly 1 (0.98 for the linear case). Reducing k_3 increased the time over which there was close agreement between the predicted and correct values, but this agreement still only occurs on the rising phase of the plasma profile.

However, two important parameters are badly predicted by this method: the peak plasma concentration and the time required to reach it. The peak plasma concentration averages 30–40% in error, while the time required to reach it is over 100% out. The results for the log normal dissolution are particularly bad, but this function is probably "too nonlinear." To be of use to the pharmaceutical industry or the clinician, both parameters would need to be predicted better. Thus, we warn against noncritical use of the method, but the method can be very useful in certain instances. To obtain reliable prediction, the mechanism of dissolution between formulations should be the same both *in vitro* and *in vivo* (as emphasized by Vaughan and Leach). Since the time over which the predictions are reliable is limited by the fastest dissolving formulation to be of use, the dissolution profiles of the formulations to be compared must be similar.

In the present analysis, we took the optimal situation in which the *in vitro* dissolution profile has been assumed to be exactly the same as occurs *in vivo*. If the time scales, however, are different—and this cannot be predicted *a priori*—the difficulties that are created may invalidate the method.

[1] The dissolution profile for the log normal law was calculated using Wagner's (3) method:

$$\frac{dA}{dt} = \frac{e^{-x^2/2}}{\sqrt{2\pi}\sigma t \log_{10} e}$$

and:

$$x = \frac{\log_{10} t - x \text{ mean}}{\sigma}$$

where x mean and σ are the mean and standard deviation of the distribution, respectively. However, rather than fixing x mean and letting σ vary as Wagner did, we fixed the ratio of x mean/σ (= 5), thus giving a more realistic spread of dissolution profiles.

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Screening Procedure for Phorbol Esters Using Brine Shrimp (*Artemia salina*) Larvae

Keyphrases □ Phorbol esters, various—toxicity to brine shrimp larvae related to cocarcinogenic potency □ Brine shrimp larvae—toxicity of various phorbol esters related to cocarcinogenic potency □ Toxicity—various phorbol esters to brine shrimp larvae, related to cocarcinogenic potency □ Cocarcinogenic potency—various phorbol esters, related to toxicity to brine shrimp larvae

To the Editor:

In the continuing search for anticancer agents of natural origin, the recently discovered antileukemic property of phorbol 12-tigliate 13-decanoate (1), from *Croton tiglium* (Euphorbiaceae), is interesting since phorbol esters are better known as irritant cocarcinogens (2). Unlike other naturally occurring anticancer compounds, intensive work has been performed on the activity of phorbol esters in two-stage carcinogenesis experiments, including their interactions with nucleic acids and their effect on the uptake of precursors of these and other macromolecules by the mammalian cell (3). Isolation of further phorbol and related esters from the plant kingdom could, in addition to providing possible new antileukemic agents, yield unique structure–activity information on the complex relationships between irritancy, cocarcinogenicity, and antileukemic activity at the cellular level.

During the fractionation of anticancer compounds from plant sources, there is often a considerable delay between isolation and receipt of biological data. Several screening

Table I—ED_{50}'s of Phorbol Esters and Two Piscicides to *A. salina* Larvae (Brine Shrimp)

Compound	ED_{50}, μg/ml	$f_{ED_{50}}$	95–100% Confidence Limits, μg/ml
Phorbol 12-tetradecanoate 13-acetate	3.8	1.96	1.9–7.4
Phorbol 12,13-didecanoate	6.8	1.96	3.5–13.3
Phorbol 12,13-dibenzoate	11.8	1.96	6.0–23.1
Phorbol	>1000	—	—
4α-Phorbol 12,13-didecanoate	>1000	—	—
Rotenone	0.5	1.71	0.3–0.9
Picrotoxin	2510	1.75	1430–4400