Table I—Calculated Inhibitory Potencies of Aryl Hydroxamic Acids

RCNHOH
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Compound	R	$^3\chi_p$	$^0\chi^v$	$^1\chi^v$	pC Found	pC Calc.
I	Pyridyl-2	3.099	5.112	2.698	3.30	3.23
II	Pyridyl-3	3.099	5.112	2.688	3.10	3.17
III	Unsubstituted phenyl	3.099	5.242	2.838	3.40	3.46
IV	2-Hydroxyphenyl	3.553	5.612	2.979	3.82	3.85
V	2-Aminophenyl	3.553	5.742	3.044	3.92	3.71
VI	3-Hydroxyphenyl	3.426	5.612	2.973	3.46	3.52
VII	3-Aminophenyl	3.426	5.742	3.038	3.46	3.38
VIII	4-Hydroxyphenyl	3.509	5.612	2.972	3.60	3.71
IX	4-Aminophenyl	3.509	5.742	3.038	3.82	3.57
X	4-Methylaminophenyl	3.917	6.665	3.499	3.48	3.79
XI	4-Dimethylaminophenyl	4.208	7.612	3.867	3.30	3.33
XII	4-Methoxyphenyl	3.917	6.573	3.361	3.30	3.24
XIII	2,3-Dihydroxyphenyl	4.145	5.982	3.119	5.10	4.60
XIV	2,4-Dihydroxyphenyl	3.873	5.982	3.113	3.60	3.92
XV	2,5-Dihydroxyphenyl	3.895	5.982	3.113	3.70	3.98
XVI	2,6-Dihydroxyphenyl	3.934	5.982	3.119	4.00	4.11
XVII	3,4-Dihydroxyphenyl	4.087	5.982	3.113	4.52	4.43
XVIII	3,5-Dihydroxyphenyl	3.664	5.982	3.107	3.40	3.40
XIX	2-Hydroxy-3-methylphenyl	4.145	6.535	3.395	3.82	4.15
XX	2-Hydroxy-4-aminophenyl	3.873	6.112	3.178	3.70	3.80
XXI	3,4-Dimethylphenyl	4.087	7.088	3.666	3.52	3.67
XXII	3,4-Diaminophenyl	4.087	6.242	3.243	4.40	4.20
XXIII	3,4-Dimethoxyphenyl	4.678	7.904	3.891	3.60	3.46
XXIV	2,4-Dichlorophenyl	3.873	7.478	3.861	3.35	3.03
XXV	3,4-Dichlorophenyl	4.087	7.478	3.861	3.60	3.54
XXVI	2,3,4-Trihydroxyphenyl	4.732	6.352	3.259	5.46	5.38
XXVII	3,4,5-Trihydroxyphenyl	4.593	6.352	3.253	5.00	5.02
XXVIII	3,4,5-Trimethoxyphenyl	5.391	9.235	4.420	4.00	4.10

and its substituents to give the quantitative differences found in enzyme inhibition values.

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Sensitive Method for Determination of Ethinyl Estradiol in Presence of Norethindrone

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Abstract □ A sensitive spectrophotofluorometric procedure for the determination of microamounts of ethinyl estradiol is described. The method is useful for the determination of ethinyl estradiol in the presence of norethindrone and common tablet excipients, especially in dissolution media.

Keyphrases □ Ethinyl estradiol—spectrophotofluorometric analysis in the presence of norethindrone and common tablet excipients □ Spectrophotofluorometry—analysis, ethinyl estradiol in the presence of norethindrone and common tablet excipients □ Dissolution rates—ethinyl estradiol in tablets, spectrophotofluorometric analysis in the presence of norethindrone and common tablet excipients

To study the dissolution rate of ethinyl estradiol and norethindrone from tablets, a sensitive procedure is needed to determine microamounts of these drugs in the presence of each other. The spectrophotometric methods described in the USP (1) for the determination of the two drugs require at least 20 tablets of each and, consequently, cannot be used to determine very small amounts of ethinyl estradiol in the dissolution medium, especially in the pres-

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Table I—Composition of Powders Using Common Excipients

Ingredient	Vial 1	Vial 2	Ingredient	Vial 3	Vial 4
Ethinyl estradiol	0[a]	0.035	Ethinyl estradiol	0	0.035
Polyethylene glycol 6000	50	50	Lactose	250	250
Stearic acid	50	50	Cornstarch	74	74
Carbomen[b]	175	175	Microcrystalline cellulose[c]	175	175
Lactose	217	217	Magnesium stearate	1	1
Magnesium stearate	8	8	Total	500	500
Total	500	500			

[a] All values are expressed in milligrams. [b] Carbopol 934. [c] Avicel.

Table II—Effect of Tablet Excipients and Reproducibility of the Fluorometric Method

Vial[a]	Amount Added, μg	Number of Determinations	Mean Amount Found ± *SD*, μg
1	0	4	0.81 ± 0.06
2	35	7	35.5 ± 0.5
3	0	4	0.40 ± 0.08
4	35	7	35.1 ± 0.7

[a] Tablet excipients are shown in Table I.

ence of norethindrone and tablet excipients. It was shown that ethinyl estradiol exhibits fluorescence in the presence of sulfuric acid (2–5).

This report describes a sensitive spectrophotofluorometric procedure for the determination of very small amounts of ethinyl estradiol in dissolution media.

EXPERIMENTAL

Reagents and Equipment—Ethinyl estradiol[1] and norethindrone[1] were used as supplied. All other solvents and reagents were analytical reagent grade. A spectrophotofluorometer[2] was used.

Standard Solution and Sample Preparation—The standard stock solution of ethinyl estradiol was prepared to contain 3.5 μg of ethinyl estradiol/ml in chloroform. To determine if the common excipients used in tablet formulations interfere with the fluorometric analysis of ethinyl estradiol, four powder mixtures (Vials 1–4) were prepared (Table I). To determine the amount of ethinyl estradiol in the dissolution medium, two tablet formulations (Vials 2 and 4) were prepared[3] using 11.9-cm concave punches and die.

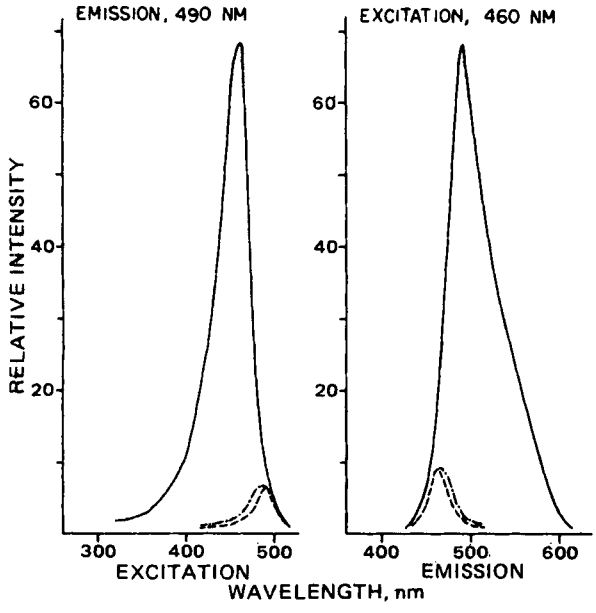

Figure 1—*Excitation and emission spectra of ethinyl estradiol. Key:* —, *ethinyl estradiol (140 ng/ml);* - · -, *norethindrone (2000 ng/ml); and* - - -, *blank.*

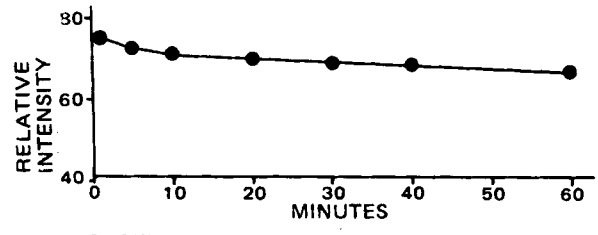

Figure 2—*Stability of fluorescence after the addition of sulfuric acid.*

Calibration Curve—Into screw-capped tubes were placed 0, 0.125, 0.25, 0.5, 1.0, and 2.0 ml of ethinyl estradiol in chloroform stock solution (3.5 μg/ml). The chloroform was removed under a nitrogen stream. The dry residue was redissolved in 0.5 ml of 4% sodium hydroxide–ethanol solution (4 g of sodium hydroxide in 100 ml of 10% ethanol–water). To this solution was added 2 ml of 80% sulfuric acid. The solution was mixed and left standing at room temperature for 30 min. The relative intensities of the fluorescence were measured at excitation and emission wavelengths of 460 and 490 nm, respectively.

Determination of Ethinyl Estradiol in Powder—Exactly 50 mg of each of the four powders (Table I) was suspended in 90 ml of water and extracted with 150 ml of chloroform. The chloroform layer was washed with 60 ml of water and dried over anhydrous sodium sulfate. Then 120 ml of the chloroform layer was evaporated to dryness under vacuum.

The residue was redissolved in 4 ml of ethanol, of which 0.5 ml was placed in a test tube and evaporated to dryness under a nitrogen stream. Ethinyl estradiol then was analyzed as described. The concentration was calculated by comparing the fluorescence of the sample to that of a known concentration treated similarly.

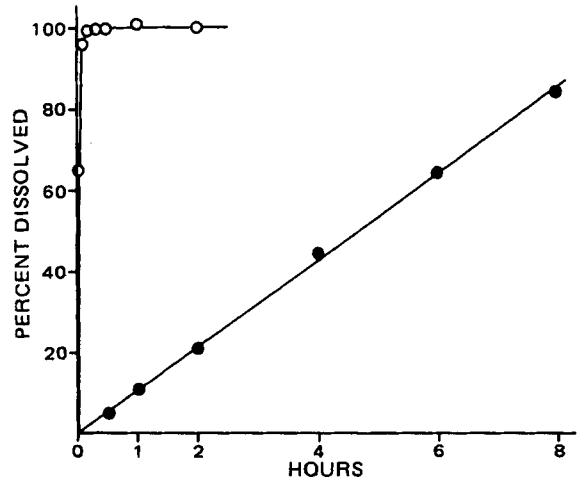

Figure 3—*Dissolution profile of ethinyl estradiol from tablets. Key:* ●, *sustained-release tablet (Vial 2); and* ○, *fast-release tablet (Vial 4).*

[1] Sigma Chemical Co.
[2] Aminco 768G, American Instrument Co., Silver Spring, Md.
[3] Stokes model 511-1 tablet machine, F. J. Stokes Corp., Philadelphia, Pa.

Determination of Ethinyl Estradiol in Dissolution Study—The release rate of ethinyl estradiol was determined at 37° using the USP rotating-basket apparatus in a dissolution medium containing 900 ml of distilled water. The basket was rotated at 100 rpm. At each sampling interval, 6 ml of the medium was withdrawn and extracted with 5 ml of chloroform. Then 4 ml of the chloroform layer was evaporated to dryness under a nitrogen stream. Ethinyl estradiol was analyzed as described.

RESULTS AND DISCUSSION

Figure 1 shows the excitation and emission spectra of ethinyl estradiol. Ethinyl estradiol exhibited strong fluorescence spectra at excitation and emission wavelengths of 460 and 490 nm, respectively. However, norethindrone did not exhibit fluorescence, even at the very high concentration of 2000 ng/ml (Fig. 1).

Figure 2 shows fluorescence stability for 1 hr after sulfuric acid addition. As can be seen in Fig. 2, the fluorescence of ethinyl estradiol was relatively stable; moreover, an excellent linearity between the fluorescence intensity and the ethinyl estradiol concentration over 17.5–280 ng/ml was observed; the minimum detection limit for the drug was ~10 ng/ml using this procedure.

The results of this study also indicate that the reproducibility of the method is excellent and that common tablet excipients do not interfere with the determination of ethinyl estradiol (Table II).

Finally, the method was valuable for the determination of small amounts of the drug in the dissolution study. Figure 3 shows the release rate of ethinyl estradiol from the sustained-release tablet (Vial 2) and the rapid-release tablet (Vial 4).

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COMMUNICATIONS

Potential Errors in Kinetic Studies of Hydrolysis of Nitrogen Mustards Based on Chloride-Ion Determination

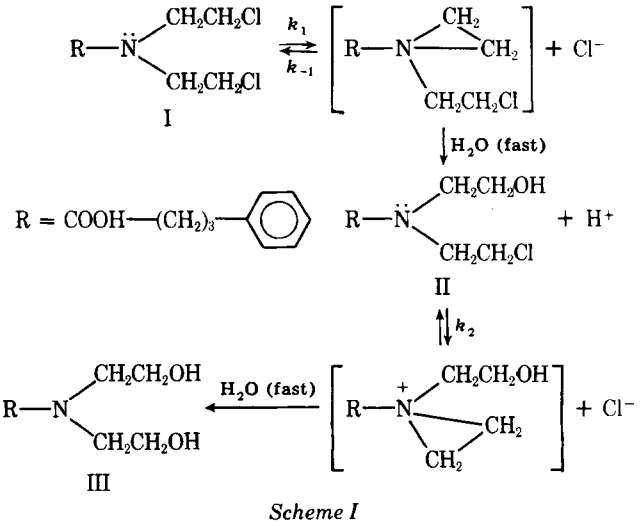
Keyphrases □ Chlorambucil—hydrolysis kinetics, potential errors based on chloride-ion determination □ Hydrolysis—chlorambucil, potential errors in kinetic studies based on chloride-ion determination □ Nitrogen mustards—chlorambucil, hydrolysis, potential errors in kinetic studies based on chloride-ion determination

To the Editor:

Chlorambucil (I), an aromatic nitrogen mustard, has been used clinically in the treatment of chronic lymphocytic leukemia and primary microglobulinemia and in the management of ovarian and testicular carcinomas (1). In aqueous solutions, I and other nitrogen mustards undergo hydrolysis, with the release of chloride ion and the formation of the cyclic ethyleneimmonium ion (1–3). This unstable cyclic intermediate then is attacked by water and other nucleophiles to yield II and other products. The same sequence then is repeated for the hydrolysis of the chloroethyl group in II (Scheme I).

Owen and Stewart recently reported (3) the kinetics of I hydrolysis using the chloride-ion measurement as a parameter for the concentration of intact I remaining. They plotted log $(Cl_\infty - Cl_t)/Cl_\infty$ *versus* time and calculated k_1, the degradation rate of I, from the apparent linear plots. In using chloride-ion measurements to obtain k_1, Owen and Stewart (3) made two important assumptions: (*a*) that the loss of I is always accompanied by the loss of two chloride ions, which is a valid assumption in the case of nitrogen mustards; and (*b*) that both chloride ions must be released simultaneously, *i.e.*, $k_2 \gg k_1$. However, there is no theoretical basis for assuming that $k_2 \gg k_1$.

The mechanism of cyclic ethyleneimmonium-ion formation should be the same for both the k_1 and k_2 steps, and there is no reason to expect that the presence of a hydroxyl function in II would make II much more unstable



Scheme I

relative to I. From a statistical consideration alone, the k_1 step probably should be faster, because there are two hydrolyzable chloro groups. Furthermore, if k_1 is not the rate-determining step, the plot of log $(Cl_\infty - Cl_t)/Cl_\infty$ *versus* time would be dependent on the relative magnitude of k_2/k_1. This relation can be shown theoretically from the classical kinetic treatment of consecutive reactions (4). Thus, for the reaction shown in Scheme II:

$$I \xrightarrow{k_1} II + Cl^- \xrightarrow{k_2} III + Cl^-$$

Scheme II

it follows that:

$$[I]_t = I_0 e^{-k_1 t} \quad \text{(Eq. 1)}$$

$$[II]_t = \frac{I_0 k_1}{(k_2 - k_1)}(e^{-k_1 t} - e^{-k_2 t}) \quad \text{(Eq. 2)}$$

$$[III]_t = \frac{I_0}{(k_2 - k_1)}[k_2(1 - e^{-k_1 t}) - k_1(1 - e^{-k_2 t})] \quad \text{(Eq. 3)}$$

$$[Cl]_t = [II]_t + 2[III]_t \quad \text{(Eq. 4)}$$

where $[I]_t$, $[II]_t$, $[III]_t$, and $[Cl]_t$ are the concentrations of

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