clarified. Therefore, the epoxidation of helenalin was undertaken. 2,3-Epoxyhelenalin (II, m.p. 217° dec.), 2,3,11,13-diepoxyhelenalin (III, m.p. 234–236°), and the dimethylamine adduct of 2,3-epoxyhelenalin (IV, m.p. 194–195°) were synthesized[1] and screened for their cytotoxicity against the growth of tissue culture cells originating from human epidermoid carcinoma of larynx (H.Ep.-2) according to a rapid microtiter method (11).

Comparison of the ED_{50} values for the cytotoxicity of the compounds listed in Table I disclosed that both the 2–3 double bond of Compound I and the 2–3 epoxide of Compound II gave equally effective cytotoxicity. The corresponding saturated compound (V) gave a 35-fold decrease in activity. Significant cytotoxicity could also be maintained when the two alkylating centers, such as the O=C—C=CH_2 system in the ketone and the lactone of helenalin, were masked by the epoxy moiety, although the diepoxide (III) was 5 times less active in comparison with helenalin (I). However, the absence of the diepoxy functionality in Compound III resulted in more than an 80-fold diminution in cytotoxicity (compare Compounds III and VI). Similar results were seen in the case of the dimethylamine adduct of 2,3-epoxyhelenalin (IV). As the epxoy group was removed, the activity was decreased (compare Compounds IV and VII). Moreover, a comparison of the activities of Compound II to Compounds I and III further indicated that the α-epoxyketonic moiety played a more important role than the α-epoxy-γ-lactonic moiety in the contribution and maintenance of the high level of cytotoxicity.

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KUO-HSIUNG LEE

Department of Medicinal Chemistry
School of Pharmacy
University of North Carolina
Chapel Hill, NC 29514

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Commencement of Basket Rotation Time as Variable in Official Dissolution Test

Keyphrases ☐ Dissolution, tablets—basket rotation time as variable in compendial dissolution test ☐ Basket rotation time—variable in compendial dissolution testing of tablets

Sir:

The dissolution characteristics of many dosage forms are now being determined by using the procedures described in either USP XVIII or NF XIII (1, 2). NF Method I and the procedure described in the USP are based on the use of a basket–stirrer assembly. This method, although official, has been criticized by a number of researchers[1] (3, 4) who found that variable results occur because of vibrational effects within the apparatus, clogging of the 40-mesh screen, and poor stirring characteristics of the assembly. During an investigation of the dissolution characteristics of several brands of chlorpromazine hydrochloride tablets, we observed another possible variable which should be considered when using this apparatus.

The compendia state that the dosage form should be placed in the basket, immersed in the dissolution medium to a point where the bottom of the basket is 2.0 cm. from the bottom of the dissolution vessel, and rotated at the speed specified in the monograph. This procedure can be varied ("Modified USP XVIII Method" in Table I) by placing the tablet in the basket, rotating the basket at the speed specified, and then immersing the rotating basket to the required depth in the dissolution medium.

Dissolution values (Table I) for one brand of chlorpromazine hydrochloride tablets were obtained by using the USP XVIII procedure and the modification of it just described. The dissolution medium was simulated gastric fluid USP (without enzyme), the total volume of medium was 900 ml., and the basket was rotated at 50 r.p.m.

The tablets were purported to contain 25.0 mg. of active ingredient. Ten tablets were assayed individually by using the procedure described in USP XVIII. Values ranged from 24.60 to 25.75 mg./tablet and indicated that there was little variability with respect to drug content.

Table I shows that drastically different results are obtained by a seemingly minor modification in the USP procedure. For example, $T_{60\%}$ values change from 23.4 to 39.3 min. but, at the same time, the modified USP method yields more reproducible results (that is, the standard deviation values are, in general, less than those reported in column 2 of Table I).

The reason for the variation in results is not at once evident but appears to be related to the mesh size of the basket (40 mesh). Studies with a 10-mesh basket yielded relatively the same values by both methods, but the values were much less than those shown in Table I. For example, the $T_{60\%}$ value for this product was ap-

[1] R. H. Blythe and W. J. Mader, "Report of the USP–NF Joint Panel on Physiological Availability," personal communication, 1969.

Table I—Dissolution Values for Chlorpromazine Hydrochloride Tablets

T Value[a]	USP XVIII Method	Modified USP XVIII Method
$T_{30\%}$	4.5 ± 1.3	6.7 ± 2.2
$T_{40\%}$	12.5 ± 4.4	20.5 ± 2.3
$T_{60\%}$	23.4 ± 7.6	39.3 ± 1.8
$T_{80\%}$	35.8 ± 10.3	60.6 ± 2.7

[a] Each T value (min.) is the mean of 10 determinations ± SD.

proximately 9 min. when a 10-mesh basket was used. Furthermore, variable results were not obtained when other brands of chlorpromazine hydrochloride tablets were tested or, if observed, the degree of variability was much less than that shown in Table I. This would appear to indicate that the inherent characteristics of the tablets tested led, in part, to the type of variability reported here.

The "correct" dissolution conditions are always difficult to establish, particularly in the absence of *in vivo* data. It is important, therefore, to standardize a procedure carefully in order to evaluate accurately various lots of the same drug product. From the results reported here, it appears that the time at which the rotation of the basket is commenced is important and that any variation from a standardized approach may yield variable results.

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B. Desta
M. Pernarowski▲
Faculty of Pharmaceutical Sciences
University of British Columbia
Vancouver 8, British Columbia
Canada

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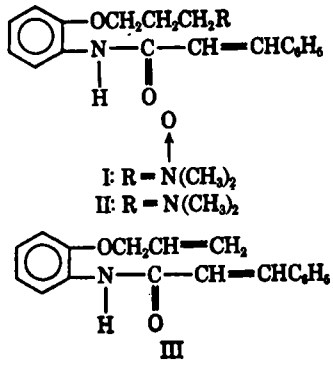
▲ To whom inquiries should be directed.

Identification of 2′-[3-(Dimethylamino)-propoxy]cinnamanilide *N*′-Oxide by Mass Spectral Thermolysis

Keyphrases □ 2′-[3-(Dimethylamino)propoxy]cinnamanilide *N*′-oxide—identified using mass spectral thermolysis □ *N*-Oxides—identification of 2′-[3-(dimethylamino)propoxy]cinnamanilide *N*′-oxide using mass spectral thermolysis □ Mass spectral thermolysis—identification, *N*′-oxide of 2′-[3-(dimethylamino)propoxy]cinnamanilide

Sir:

The occurrence of *N*-oxides as natural products or as metabolites of pharmacologically active compounds



III

was reviewed previously (1). The only mass spectral studies of *N*-oxides have been those of aromatic *N*-oxides that usually exhibit the M^+-16 ion, instead of the M^+, as the highest mass ion (2). Mass spectrometry was used to elucidate the structures of some thiopropyldimethylamino-*N*-oxide metabolites (3). In this report, these observations are extended to the thermolysis of 2′-[3-(dimethylamino)propoxy]cinnamanilide *N*′-oxide (I).

Since volatilization of the *N*-oxide base, I (mol. wt. 340), occurs very near its thermolysis point, the spectra obtained at temperatures below 150° were extremely weak and would not normally suffice to establish the structure of metabolites in the presence of the usual artifacts. Generally, the similarity in the mass spectra[1] of *N*-oxides and of free amines would make it difficult to assign structures to *N*-oxide metabolites. However, variation of the ion-source temperature does permit differentiation of amines and their *N*-oxides. At 155° or higher, I showed no M^+, and the M^+-16 ion, though still present, was diminished in intensity relative to the *m/e* 279 ion (Table I). The composition of the *m/e* 279 (M^+-61) ion corresponds to the olefin, III, formed by the Cope elimination of an *N*-oxide (5).

The *N*-oxide hydrochloride, I-HCl, shows the M^+-16 ion, the *m/e* 58 ion, and a temperature-dependent ion at *m/e* 279. At 185°, fragment ions of I-HCl are present at M^+-18 and M^+-30 (the former being a dehydration product) but are absent at higher temperatures. At temperatures >185°, the *m/e* 279 ion is more intense. However, the persistence of the M^+-16 ion, coupled with the increase in intensity of the *m/e* 58 ion, demonstrates that the thermolysis of I-HCl yields more II (mol. wt. 324) than does the thermolysis of I.

Differential thermal and thermal gravimetric analyses[2] also show the difference in the thermolysis of the hydrochloride and the free base of the *N*-oxide. The hydrochloride, I-HCl, melts in two exothermal steps at 186 and 191°, with a total loss of 7% of its weight. Since the loss of oxygen requires a weight loss of 4.3%, and the Cope elimination product requires a weight loss of 12%, the intermediate value for weight loss demonstrates that a mixed-product thermolysis can occur and substantiates the mass spectral results, which show that various thermolytic products were formed. But the free base of the *N*-oxide, I, showed an endotherm at

[1] The equipment and experimental technique for obtaining the electron-impact spectra were described in *Reference 4*.
[2] Performed on a Dupont analyzer.