Wilforine

Sir:

Recently, Hayashi and Mitsuhashi (1) reported on the separation and structure determination of a new pregnane derivative from *Cynanchum wilfordi*, to which they assigned the trivial name wilforine.

An alkaloid of as yet undetermined structure was isolated by Beroza (2) in 1952 from the unrelated plant *Tripterygium wilfordii*, which he named wilforine. The same alkaloid was more recently isolated from *Maytenus senegalensis* (3).

Since these two substances are obviously dissimilar, the identical naming of them has created confusion in the literature. The product from *C. wilfordi* should be renamed by Hayashi and Mitsuhashi in subsequent publications.

(1) K. Hayashi and H. Mitsuhashi, *Chem. Pharm. Bull.*, **20**, 2065 (1972).
(2) M. Beroza, *J. Amer. Chem. Soc.*, **74**, 3656(1952).
(3) M. Tin-Wa, N. R. Farnsworth, H. H. S. Fong, R. N. Blomster, J. Trojánek, D. J. Abraham, G. J. Persinos, and O. B. Dokosi, *Lloydia*, **34**, 79(1971).

NORMAN R. FARNSWORTH
Department of Pharmacognosy
and Pharmacology
College of Pharmacy
University of Illinois
at the Medical Center
Chicago, IL 60612

Antitumor Agents V: Effect of Epoxidation on Cytotoxicity of Helenalin-Related Derivatives

Sir:

In connection with our study of the structure–activity relationships among helenalin (I)-related sesquiterpene lactones for cytotoxic or antitumor activity (1–3), we wish to report a preliminary account of the effect of epoxidation on cytotoxicity of helenalin-related derivatives.

The epoxy function is a structural feature commonly found in many naturally occurring sesquiterpene

Table I—Cytotoxicity of Helenalin-Related Derivatives

Number	Compound	Reference	ED$_{50}$, mcg./ml. (H.Ep.-2)
I	Helenalin	1–3	0.10
II	2,3-Epoxyhelenalin	—[a]	0.11
III	2,3,11,13-Diepoxyhelenalin	—[a]	0.50
IV	2,3-Epoxyhelenalin dimethyl-amine adduct	—[a]	1.36
V	2,3-Dihydrohelenalin	2	3.84
VI	2,3,11,13-Tetrahydro-helenalin	2	>40
VII	2,3-Dihydrohelenalin dimethylamine adduct	2	6.04

[a] K. H. Lee, S. H. Kim, H. Furukawa, C. Piantadosi, and E. S. Huang, unpublished data.

lactones. It is well known that certain classes of synthetic compounds (4), as well as naturally occurring substances, owe their antitumor or cytotoxic activity to the introduction of the diepoxide or triepoxide functionality; *e.g.*, the cyclohexane diepoxide, crotepoxide (5); the sesquiterpene dilactone diepoxide, mikanolide (6, 7); and the diterpene triepoxides, triptolide and tripdiolide (8). With this in mind, it has been inferred that one important factor governing the cytotoxicity of the sesquiterpene lactones could be due to the introduction of the epoxy group. The epoxy group might act as a second alkylating function in addition to the essential alkylating center, the α-methylene-γ-lactone moiety (1, 9, 10). However, it was suggested (6) that cytotoxicity appears to be independent of the presence or absence of an epoxy group, although this conclusion was based upon only a small number of examples.

We felt that the role of the epoxide function with respect to the effect upon cytotoxicity should be further
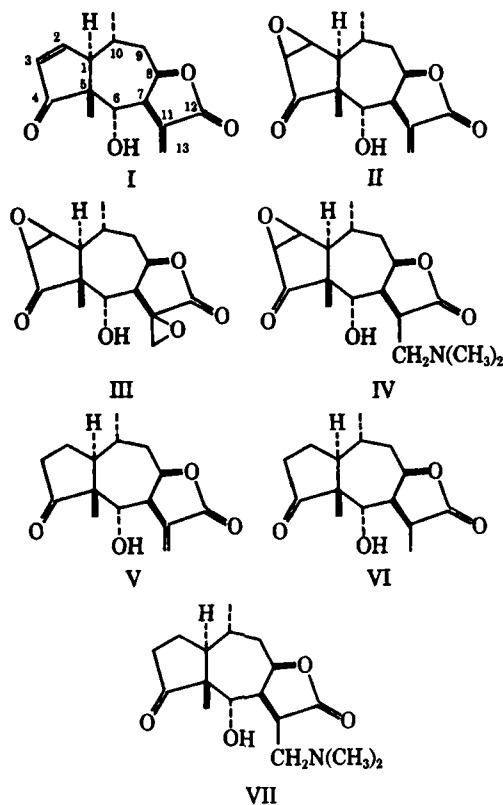
clarified. Therefore, the epoxidation of helenalin was undertaken. 2,3-Epoxyhelenalin (II, m.p. 217° dec.), 2,3,11,13-diepoxyhelenalin (III, m.p. 234–236°), and the dimethylamine adduct of 2,3-epoxyhelenalin (IV, m.p. 194–195°) were synthesized[1] and screened for their cytotoxicity against the growth 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

[1] K. H. Lee, S. H. Kim, H. Furukawa, C. Piantadosi, and E. S. Huang, unpublished data.

Commencement of Basket Rotation Time as Variable in Official Dissolution Test

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.