

microscopically and while a histological study was not made, gross microscopic examination showed no differences from normal tissue.

Each of three adult male subjects was given ursolic acid orally in doses of 0.5 Gm. daily (20 mg. per Kg. body weight) over a 3-day period. No symptoms or discomfort of any kind were noted. We may conclude, consequently, that ursolic acid in moderate amounts is innocuous to humans.

#### SUMMARY

1. According to the literature (9) ursolic acid in a saturated solution of its sodium salt is toxic to fish.

2. Ursolic acid was found to be non-toxic to rats, guinea pigs, chickens, rabbits and humans.

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## A Colorimetric Method for the Assay of Diethylstilbestrol\*

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Various methods have been described in the literature (1, 2, 3) for the quantitative estimation of diethylstilbestrol. These methods have the disadvantage of a narrow range of applicability, or they require apparatus not generally available in control laboratories. An attempt was therefore made to devise a colorimetric method which would be applicable to the estimation of relatively small amounts of diethylstilbestrol present in pharmaceutical preparations.

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It was found that the xanthoproteic reaction was suitable for this purpose and in this paper is presented a method which is simple, rapid and accurate, for the estimation of diethylstilbestrol in tablet, ampul and suppository preparations.

It should be emphasized that the method to be described is applicable only to those preparations in which diethylstilbestrol is the only phenyl derivative present.

#### EXPERIMENTAL

*The Color Test.*—Transfer an aliquot of a methanol solution of pure diethylstilbestrol to a test-tube graduated at 16 cc. Remove the methanol in a boiling water bath with the aid of vacuum. To the residue add 0.75 cc. of concentrated nitric acid (sp. gr. 1.42) and place the tube in a boiling water bath for ten minutes. Cool the tube to room temperature and add 4 cc. of 10% ammonium hydroxide. Cool again to room temperature and dilute to 16 cc. with water.

Experiments showed that the color produced by the reaction was stable over long periods of time and was unaffected by various concentrations of reagents over a wide range. The 10-minute period of heating was found to ensure maximum color development.

Using this method on amounts from 0.25 mg. to 1.75 mg. of diethylstilbestrol the yellow colors produced were found to be linear functions of the concentrations of diethylstilbestrol present when measured in a B. & L. Duboscq Colorimeter.

Attention was next turned to the effect on the color reaction of those substances commonly used as tablet excipients, *e. g.*, starch, lactose, acacia, magnesium stearate, calcium phosphate. It was found that when a methanol extract of various known amounts of diethylstilbestrol was made in the presence of a proportionate amount of a mixture of these excipients and compared with a methanol solution of 0.5 mg. diethylstilbestrol as standard, practically quantitative results were obtained.

The following procedure was therefore applied to the assay of tablets.

Weigh a number of tablets equal to 5 mg. to 10 mg. of diethylstilbestrol and determine the average weight of one tablet. Powder the tablets and take a weight of the powder equal to approximately 5 mg. of diethylstilbestrol. Macerate the powder in 10 cc. of methanol for 15 minutes with the aid of a glass rod. Filter and transfer an aliquot equal to 0.5 mg. of diethylstilbestrol to a test-tube graduated at 16 cc. Treat as described under Color Test, and compare the color to that given by 0.5 mg. of a standard prepared in the same way.

Using this method in a considerable number of assays on carefully prepared tablets an average deviation of  $\approx 1\%$  was obtained with a maximum deviation of  $\approx 5\%$  in only two instances.

Table I.—Comparison of the Effect of Tablet Excipients

Sample <sup>a</sup>	Standard, Mg.	Control Reading	Sample <sup>b</sup> Reading	Theoretical, Mg.	Found, Mg.	Recovery, %
1	0.5	20.0	10.0	1.00	1.00	100
2	0.5	20.0	13.26	0.75	0.754	100.5
3	0.5	20.0	20.18	0.50	0.497	99.5
4	0.5	20.0	29.92	0.25	0.251	100.5

<sup>a</sup> Each sample contained 65 mg. of excipient mixture.

<sup>b</sup> The sample readings shown are the average of 5 readings.

*Assay Method for Solutions of Diethylstilbestrol in Oil.*—Solutions of this nature are usually prepared by dissolving the diethylstilbestrol in a vegetable oil such as sesame oil.

For experimental purposes such a solution was prepared by dissolving 50 mg. of diethylstilbestrol in 50 cc. of sesame oil. Different methods of extraction were tried and the following one was found to give quantitative results.

Place sufficient of the oil to give approximately 5 mg. of diethylstilbestrol in a small separatory funnel and add 30 cc. of light petroleum ether. Extract three times with *N*/1 sodium hydroxide, using 15 cc., 10 cc., 10 cc. Combine the alkaline extracts, acidify with 10% sulfuric acid and extract three times with ether, using 20 cc., 15 cc., 10 cc. Combine the ether extracts and evaporate to dryness. Dissolve the residue in 10 cc. of methanol and apply the color reaction to an aliquot representing 0.5 mg. of diethylstilbestrol as described under Color Test.

Results obtained with this method showed an average deviation of  $\pm 1\%$ .

*Assay Method for Suppositories of Diethylstilbestrol.*

—The suppositories used in these experiments were of the glycerine-gelatine type. Several methods of extraction were tried and finally the following one was used.

Place a number of suppositories equal to approximately 5 mg. of diethylstilbestrol in a separatory funnel, add 50 cc. of hot water and agitate until dissolved. Cool and extract three times with ether using 20 cc., 15 cc., 10 cc. Combine ether extracts and extract twice with 15 cc. of *N*/1 sodium hydroxide. Acidify the combined alkaline extracts with 10% sulfuric acid and extract three times with ether using 15 cc., 10 cc., 10 cc. Combine the ether extracts and evaporate to dryness. Dissolve the residue in 10 cc. of methanol and take an aliquot equal to 0.5 mg. of diethylstilbestrol and treat as described under the Color Test.

Results obtained with this method showed deviations of 5%.

#### SUMMARY

A color reaction for the quantitative estimation of diethylstilbestrol in tablets, ampuls and suppositories has been described.

This reaction is not specific; it is applicable to preparations in which stilbestrol is known to be the only phenyl derivative present.

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## A Critical Study of the Physico-Chemical Properties of Tyrolean Oil *Pinus Pumilio* Haenke (Oil *Pinus Montana* Miller) Fam. Pinaceae and Oil *Abies Alba* Miller (Oil *Abies Pectinata* D. C.) Fam. Pinaceae\*

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During recent years, the properties of shipments of guaranteed purity of Oil *Pinus Pumilio* Haenke (Oil *Pinus Montana* Miller) (Fam. Pinaceae) and Oil *Abies Alba* Miller (Oil *Abies Pectinata* D. C.) (Fam. Pinaceae) received from the Tyrol were found to differ considerably from the limits set forth by the standard accepted literature (1) and in the case of Oil *Pinus Pumilio* (Oil of Dwarf Pine Needles) from the standards of the United States Pharmacopoeia. This research was undertaken to study the physical and chemical properties of authentic samples of Oil *Pinus Pumilio* and Oil *Abies Alba* from the Tyrol which were distilled under the instruction of our Research Department in different locations, at different times and under different conditions of weather, plant material, etc.

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