TINCTURE AND FLUIDEXTRACT OF DIGITALIS.*

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The most pressing problem connected with preparations of digitalis at the present time is that of securing stability. For some reason, as yet but partially understood, preparations of this drug deteriorate rather rapidly for the first few months, then come to a condition of relative stability and deteriorate much more slowly. The relative rate of change is not uniform either in the initial stage or in the later.

In so potent a drug uncertainty of strength is a serious obstacle, and the stabilizing of its preparations becomes highly important. Fortunately, the establishment of the fact that the activity resides entirely in the glucosides simplifies the problem to a considerable extent.

Since glucosides undergo hydrolysis quite easily and all liquid preparations of digitalis contain water in varying amounts, the first suggestion that occurs is to find some way to prevent hydrolysis.

This action may be due to a hydrolytic enzyme coexisting in the drug which produces the reaction, or it may be due to an inherent instability of the glucoside in the presence of even a small amount of water. Both factors were considered in the following experiments.

In the fall of 1930, fifty pounds of the fresh first-year leaves of *Digitalis purpurea*, grown at the Parkedale farm near Rochester, Mich., were cut and divided into two portions. (The weight here is that of the fresh, undried leaves.) Twenty-five pounds were covered immediately with alcohol, ten gallons of alcohol being used, shipped to the Detroit laboratory in this condition, and on the day after cutting, the mixture was heated to the boiling point of alcohol for half an hour, the object being to destroy any hydrolytic enzyme that might be present in the fresh drug.

The other twenty-five pounds were placed in a vacuum dryer and dried as rapidly as possible to a practically constant weight, at a temperature of about 40° C.

The alcohol, after cooling, was drained from the now sterilized leaves, the leaves pressed, then dried in the vacuum dryer. These leaves dried much more quickly, as was expected, since most of the water had already been displaced by alcohol. The alcoholic liquid recovered measured 35130 cc. (9.28 gals.), tested 74.9% of alcohol by volume, and yielded 1.81% extractive or a total of 634.9 Gm.

The dried (sterilized) leaves weighed 1845 Gm. (4.067 lbs.) to which the extractive from the alcohol solution is added, making a total of 2480 Gm. (or 5.467 lbs.). The alcoholic liquid was then concentrated by distillation *in vacuo* so that 1 cc. would represent 2 Gm. of drug. All preparations made were then prepared by calculation so that the amount of drug used, plus an equivalent of the alcoholic concentrate would represent an equivalent of normal air-dry drug.

Before extraction the drug was defatted by extracting with petroleum ether. The weight, after drying was 1788 Gm. (3.92 lbs.) showing 3.09% of fat extracted.

From the unsterilized leaves there was obtained 2535 Gm. of dry leaves (5.588 lbs.) which after grinding was extracted with petroleum ether and again dried, yielding 2462 Gm. (5.42 lbs.).

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We thus from 25 lbs. (11,440 Gm.) of fresh leaves obtained 2462 Gm. of defatted dry powder for extraction in one case, and 1788 Gm. of sterilized defatted powder in the other case, to which is to be added 635 Gm. of extractive taken out by the alcohol used in sterilizing making a total of 2423 Gm. of dry fat-free material.

The powdered leaves were then used in the proportion of 2462:1788::1:0.726 or about three-fourths as much of the sterilized leaves as of the unsterilized, since to the former one cc. of the concentrated alcoholic liquid was added to the product for each 2 Gm. of drug used to restore the extractive previously taken out. From each drug there was then prepared three tinctures and three fluidextracts in the usual manner, using 77% alcohol (alcohol 4 volumes—water 1 volume) as the menstruum.

One tincture from each drug represented the official preparation as now formulated, one was adjusted to a lower p_H, and one was saturated with anhydrous sodium acetate. Each preparation was assayed by the M. L. D. frog method when first made, and again after standing about six months.

The results are as follows:

U. S. P. Tincture of Unsterilized Drug.

p_H = 5.65
Assay, fresh = 300%
Assay, after 5 mos. = 140%
Loss = 53%

U. S. P. Tincture of Sterilized Drug.

p_H = 5.50
Assay, fresh = 125%
Assay, after 5 mos. = 90%
Loss = 28%

Corresponding tinctures to which were added hydrochloric acid.

Unsterilized Drug.

p_H = 4.01
Assay, fresh = 125%
Assay, after 5 mos. = 110%
Loss = 12%

Sterilized Drug.

p_H = 4.00
Assay, fresh = 260%
Assay, after 5 mos. = 175%
Loss = 33%

Corresponding tinctures containing anhydrous sodium acetate and acetic acid.

Unsterilized Drug.

p_H = 5.00
Assay, fresh = 110%
Assay, after 6 mos. = 125%
No loss

Sterilized Drug.

p_H = 4.90
Assay, fresh = 125%
Assay, after 6 mos. = 125%
No loss

The idea of using anhydrous sodium acetate was that it might act by absorption of the water in the menstruum and hinder or prevent hydrolysis of the glucosides. It was found by experiment that when a 77.4% alcohol was saturated with anhydrous sodium acetate the solution contained 5.6 Gm. of the salt in 100 cc. of solution, and had a p_H of 8.2. Since an alkaline reaction in digitalis preparations is detrimental to activity enough glacial acetic acid was also added to bring the p_H to correspond approximately with that of the tinctures containing hydrochloric acid. One hundred and twenty cc. of glacial acetic acid was found to be necessary per 1000 cc. of tincture. This necessitated stopping the percolation at four-fifths the normal yield, since each 100 cc. of tincture contained 6 Gm. of anhydrous sodium acetate and 12 cc. of glacial acetic acid.

The corresponding fluidextracts show the following results:

N. F. Fluidextract from Unsterilized Drug.

p_H = 5.48
Assay, fresh = 80%
Assay, after 6 mos. = 55%
Loss = 30%

N. F. Fluidextract from Sterilized Drug.

p_H = 5.50
Assay, fresh = 115%
Assay, after 6 mos. = 70%
Loss = 40%

N. F. Fluidextract with HCl, Unsterilized.

p_H = 4.01
Assay, fresh = 60%
Assay, after 6 mos. = 60%
No loss

N. F. Fluidextract with HCl, Sterilized.

p_H = 3.85
Assay, fresh = 100%
Assay, after 6 mos. = 60%
Loss 40%

N. F. Fluidextract + Acetic Acid and Acetate, Unsterilized.

p_H = 5.50
Assay, fresh = 50%
Assay, after 6 mos. = 70%
No loss

N. F. Fluidextract + Acetic Acid and Acetate, Sterilized.

p_H = 4.80
Assay, fresh = 100%
Assay, after 6 mos. = 60%
Loss = 40%

Since the sterilization of the fresh leaves by treatment with hot alcohol introduces commercial difficulties which are serious, further experiments were made to ascertain the comparative effect of sterilization on the dried (unsterilized) leaf.

A tincture was prepared as follows: To 100 Gm. of the dried digitalis was added 100 cc. of alcohol (95%), the mixture allowed to stand over night in a stoppered flask, then heated under a reflux condenser in a boiling water-bath for 30 minutes. The mixture was then allowed to cool, 25 cc. of water added and well mixed, again macerated over night, then packed in a percolator and extracted with 77% alcohol until 1000 cc. of tincture was obtained. To half of this tincture or 500 cc. there was added 4 cc. of concentrated hydrochloric acid, to bring the p_H down from 5.43 to 3.99.

Assay, fresh = 250%
Assay, after 6 mos. = 220%
Loss = 12%

To the other 500 cc. was added 1.05 cc. of 50 per cent hypophosphorous acid, which reduced the p_H to 3.95.

Assay, fresh = 275%
Assay, after 6 mos. = 125%
Loss = 52%

Another tincture was made as follows: To 50 Gm. of the dried digitalis was added 50 cc. of 87% alcohol (alcohol 9 vols., water 1 vol.) well mixed and allowed to stand in a stoppered flask over night. This was then heated in a boiling water-bath under a reflux condenser for 15 minutes, cooled, packed in a percolator and after maceration extracted with 87% alcohol to obtain 500 cc. of tincture. To this was added 0.75 cc. of 50% hypophosphorous acid, which produced a p_H of 3.55.

Assay, fresh = 250%
Assay, after 6 mos. = 125%
Loss = 50%

SUMMARY.

Sterilization of the leaves by hot alcohol appears to increase the stability of the tincture. If applied to the undried leaves exposure to hot alcohol vapors would undoubtedly be more practical than maceration in boiling alcohol, but the results indicate that the application of hot alcohol to the dried leaves may be quite as effective and more economical. The results thus far are not at all conclusive and the subject will be continued.

On the fluidextract all results are erratic and no conclusions can be drawn. Undoubtedly the heat and exposure during the concentration of the weak percolate affects the activity to some extent and introduces a factor which cannot be easily controlled. It also opens the question whether the fluidextract is sufficiently reliable to warrant its continuation.

The acidity also appears to be a factor in stability. The addition of hydrochloric acid to secure a p_H of about 4.0 favored stability in three cases out of five. Whether more acid would increase the stability we have yet to learn. Hypophosphorous acid proved to be more of a detriment than a help.

The use of anhydrous sodium acetate as an anti-hydrolytic agent shows some very interesting results. The only preparations which show no loss in six months are those that contain this agent. But there is an unaccountable low initial assay, though all these samples except one of the fluidextract are above the standard. Since these preparations contain about 12 per cent of acetic acid this may impair the accuracy of the assay when injected into the lymph sac of the frog. This question as well as further experiments on the influence of sodium acetate will be followed up. It is planned to continue the investigation with the hope of securing more stable preparations of Digitalis for the next U. S. P. and N. F. Coöperative work on this line is invited from any who may be interested.

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THE INTERNATIONAL CONGRESS OF PURE AND APPLIED CHEMISTRY.

The Ninth International Congress of Pure and Applied Chemistry will be held in Madrid from April 3rd to 10th. The officers of the Congress are: J. R. Mourelo, *vice-president* of the Academy of Sciences and professor Emeritus of the School of Arts, *honorary president;* O. Fernandez, *professor* at the University of Madrid, member of the Academy of Sciences and dean of the Academy of Pharmacy, *president;* and E. Moles, professor at the university and at the National Institute of Chemistry and Physics and president of the Spanish Society of Chemistry and Physics, *general secretary.*

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