Comparison of Extractive Procedures for Digitalis

By BERNARD R. LETASSY† and C. LEE HUYCK‡

The shearing action of the Premier colloid mill and the Eppenbach Homo-Mixer was applied to a mixture of digitalis powder and its extracting liquid in order to determine whether these pieces of equipment might be superior to the percolator for extraction of the active principles. The official method was used as the control procedure and simple agitation as an intermediate procedure. Data presented show order of effectiveness.

A MORE efficient extraction of the active principles of digitalis has been the target of numerous investigators. Bay and Gisvold (1) destroyed the enzymes in fresh digitalis leaves by heating the ground leaves to 70° before attempting extraction. In a later investigation Hopponen and Gisvold (2) found that quick freezing of the freshly harvested digitalis leaves retarded enzyme action and kept the glycoside content at a maximum until extracted. In an extension of this work (3) a maximum yield was obtained when the aqueous extraction represented two per cent of the drug on a dry leaf weight basis. Eighty-seven per cent of the activity could be extracted in this way. Aqueous extraction possesses several disadvantages, however. One disadvantage is hydrolysis of the active principle with a subsequent lower yield of glycosides. Another disadvantage is the glycosides are less soluble in water than they are in alcohol.

Kušević (4) made a comparative study of maceration, percolation, agitation, and digestion in an attempt to determine whether a more effective method for extraction of digitalis could be found. The digestion method with dilute alcohol at 60° gave the best results of the methods tested.

A somewhat different approach to the problem of extraction was taken by Dean, *et al.* (5), who demonstrated that it is possible to prepare tinctures in a few minutes, whereas the conventional methods require several days. This was accomplished by passing a suspension of the powdered drug in menstruum through a colloid mill. Using belladonna leaf, a 5-minute maceration yielded 37.2 per cent of the desired components. The percentage of yield increased to 99.5 per cent when the mixture of leaf and menstruum was circulated in a colloid mill continuously for 5 minutes.

EXPERIMENTAL

Using the milling method described by Dean, *et al.* (5), as a guide an attempt was made to increase the yield obtained by the official method for extraction of digitalis glycosides. In addition to the colloid mill, three other pieces of equipment were utilized: the Eppenbach Homo-Mixer, simple agitation in a screw-top jar; and the percolator (by the U.S.P. method which served as a control). Agitation in a jar provided an intermediate method to determine whether simple agitation had any effect on improving the extraction of the macerated leaf.

Procedure.—Four 100-gram portions of 80-mesh *Digitalis purpurea* powder were made evenly and distinctly damp with the menstruum (4 volumes alcohol and 1 volume water) and allowed to stand for 15 minutes. The control portion was packed in a cylindrical percolator. All four portions were allowed to macerate for 24 hours with additional menstruum.

At the end of the maceration period a suspension of the first portion was passed through the Premier colloid mill, type U-B7, using a setting of 0.005 inch between rotor and stator. The suspension was filtered and the marc was washed with menstruum until the filtrate measured 1,000 ml. A suspension of the second portion was subjected to the Eppenbach Homo-Mixer, type CS, for 5 minutes at maximum speed without loss of liquid and filtered through paper. The marc was washed with menstruum to volume. The third portion was shaken vigorously for 5 minutes in a one-gallon screw-top jar and filtered through paper. Likewise the marc was washed with menstruum to volume.

In order to insure accuracy, the entire process was repeated with another four 100-Gm. portions of the same batch of powder. The resulting tinctures were assayed by the chemical method of Bell and Krantz (6).

TABLE I.—PERCENTAGE STRENGTH OF TINCTURES PREPARED BY THE VARIOUS METHODS OF EXTRACTION IN TERMS OF U.S.P. REFERENCE STANDARD TINCTURE

Method	1st	2nd	Average
U.S.P. XV	88.5	89.7	89.1
Eppenbach Homo-Mixer	78.8	65.0	71.9
Premier colloid mill	73.5	69.0	71.2
Simple agitation	63.4	64.0	63.7

Table I reveals that percolation of the macerated digitalis leaf is superior to the other extraction methods. Probably the most important factor in favor of the percolation method is the fact that fresh menstruum is continually passing around the particles of the drug. Although this factor also took place in the modified methods where the marc was continually washed on the filter paper, it took place less efficiently. Table I also reveals the order in which the glycosides were extracted when percolation was not a factor. The Homo-Mixer gave approximately the same results as the colloid mill, and both were better than simple agitation.

SUMMARY

An attempt to increase the yield of digitalis glycosides by applying high speed shearing to a mixture of powdered drug and menstruum in place of percolation by the U.S.P. method was made. Shearing was obtained by two pieces of equipment

Received October 4, 1962, from the St. Louis College of Pharmacy, St. Louis, Mo.
Accepted for publication October 29, 1962.
† Present address: Chief Pharmacist, Poplar Bluff Hospital, Poplar Bluff, Mo.
‡ Present address: Eaton Laboratories Division of The Norwich Pharmacal Co., Norwich, N. Y.

commonly found in the manufacturing laboratory: the Premier colloid mill and the Eppenbach Homo-Mixer. Simple agitation in a screw-top jar was used as an intermediate procedure. Duplicate experiments (using these pieces of equipment) show the official method of extraction of digitalis (using the percolator) to be superior to methods using the other pieces of equipment tested.

REFERENCES

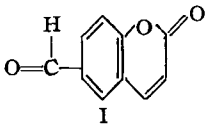
(1) Bay, G., and Gisvold, O., THIS JOURNAL, 37, 314 (1948).
(2) Hopponen, R. E., and Gisvold, O., *ibid.*, 41, 146(1952).
(3) King, R. E., and Gisvold, O., *ibid.*, 39, 109(1950).
(4) Kuševič, V., *Farm. Glasnik.*, 12, 243(1956); through *Chem. Abstr.*, 50, 17315g(1956).
(5) Dean, S. J., Brodie, D. C., Brochmann-Hanssen, E., and Riegelman, S., THIS JOURNAL, 42, 88(1953).
(6) Bell, F. K., and Krantz, J. C., *J. Pharmacol. Exptl. Therap.*, 83, 213(1945).

Antitubercular Activity of Some Aromatic Aldehyde and Ketone Derivatives

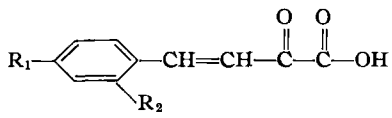
By AJIT K. DATTA† and T. C. DANIELS

The semicarbazones, para-nitrobenzoyl hydrazones and isonicotinyl hydrazones of the unsubstituted and three substituted 4-phenyl-2-oxo-3-butenoic acid and 6-formyl-1,2-benzpyrone were prepared and the toxicities and *in vitro* antitubercular activities measured. Three isonicotinyl hydrazones show the same order of antitubercular activity as isonicotinic acid hydrazide.

THE WELL KNOWN antitubercular activity of thiosemicarbazones, *p*-nitrobenzoyl hydrazones, and isonicotinyl hydrazones of a number of substituted and α,β-unsaturated aromatic aldehydes (1, 6) and ketones (2) made it of interest to prepare the corresponding derivatives of 6-formyl-1,2-benzpyrone (I) and of 4-phenyl-2-oxo-3-butenoic acid (II; R_1, R_2 = H), the latter possessing the additional advantage of affording water-soluble salts.



I



II

In view of the interesting antitubercular activity of the isonicotinyl hydrazone of II, the corresponding *o*-chloro, *o*-methoxy, and *p*-methoxy substituted derivatives of II were prepared for comparison and are described in Table I.

In vitro antitubercular activity was determined against *Mycobacterium tuberculosis* var. *hominus* strain H 37 RV by the serial dilution technique, using the modified Dubos medium (3). The results are shown in Table II.

Acute toxicity of the compounds was determined using intraperitoneal injections of suspensions in 6% acacia in Webster strain mice of both sexes (average weight 20 Gm.). The results are given in Table III.

EXPERIMENTAL[1]

6-Formyl-1,2-benzpyrone.—Prepared by the method of Sen and Chakravarti (4), the compound melted at 189° (lit. m.p. 187–189°) (4).

6-Formyl-1,2-benzpyrone Thiosemicarbazone.—A mixture of 0.8 Gm. of 6-formyl-1,2-benzpyrone in 35 ml. of 80% ethanol, 0.45 Gm. of thiosemicarbazide, and 1 Gm. of sodium acetate was refluxed on a steam cone for 1 hour. The reaction product was recrystallized from dilute alcohol. One gram (85% yield) of a cream-colored crystalline product was obtained. The compound sublimes without melting at 300°.

Anal.—Calcd. for $C_{11}H_9N_3O_2S$: N, 17.0. Found: N, 16.79.

6-Formyl-1,2-benzpyrone *p*-Nitrobenzoyl Hydrazone.—A mixture of 0.9 Gm. of 6-formyl-1,2-benzpyrone in 35 ml. of 80% ethanol and 0.9 Gm. of *p*-nitrobenzoyl hydrazide in 20 ml. of ethanol was refluxed for 1.5 hours on a steam bath. The reaction product was recrystallized from 30% alcohol from which 1.5 Gm. (90% yield) of lemon-yellow crystals was obtained, m.p. 314–315°.

Anal.—Calcd. for $C_{17}H_{11}N_3O_5$: N, 12.46. Found: 12.7.

6-Formyl-1,2-benzpyrone Isonicotinyl Hydrazone.—A 0.7-Gm. quantity of isonicotinic acid hydrazide in 10 ml. of distilled water was added to a solution of 0.9 Gm. of 6-formyl-1,2-benzpyrone in 35 ml. of 80% ethanol. The mixture was heated on a steam bath; white crystals started separating immediately. The crude product was recrystallized from water. The product weighed 1.4 Gm. (92% yield) and melted at 284–285°.

Anal.—Calcd. for $C_{16}H_{10}N_3O$: N, 14.33. Found: N, 14.53.

Received October 16, 1962, from the Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco.
Accepted for publication January 18, 1963.
The authors are indebted to Professor John C. Craig for his helpful advice and suggestions.
This paper is a portion of a thesis submitted by Ajit K. Datta to the Graduate Division, University of California, in partial fulfillment of Doctor of Philosophy degree requirements.

† Present address: Hoechst Pharmaceutical Co., Scientific Department, Calcutta 13, India.

[1] Microanalysis of the compounds reported was carried out by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley.