Identification and Evaluation of Botanical Extracts by Paper Chromatography

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The problem of identification of botanical drug extracts is enormous in view of the large variety of botanical species as well as other factors affecting the biosynthesis of plant constituents. This is particularly true for those drugs which do not have a pharmacologically active ingredient or chemical component which can be assayed for in a routine manner. A rapid method is proposed which utilizes an ascending paper chromatographic technique with slight modification, ultraviolet light for observation, and the use of appropriate chromogenic spray reagents. This procedure produces a chromatogram consisting of base, stem, cone, and solvent front regions. The resulting characteristic picture, when viewed as a whole in comparison with a standard or control sample, can then be used for the identification and evaluation of the drug extract.

THE IDENTIFICATION of botanical drugs is accomplished by a variety of analytical and biochemical methods. Generally speaking, these have been based on both macroscopic and microscopic pharmacognostical descriptions, color reactions, assay procedures for the determination of chemical constituents as well as specific physiological responses observed through biological assay techniques.

Due to the complexity of most drugs, these procedures are generally lengthy and rather intricate so that identification often is based solely on physical characteristics. In the case of extracts of these drugs, so often indistinguishable in such a form, identification poses an even greater problem. As many extracts, fluidextracts, tinctures, and similar preparations are daily analyzed in our laboratories, our concern was more immediate and led us to devise a rapid identification method. The natural choice was paper chromatography. The method described using this technique has met with good success and is now being used as a routine control procedure.

Accounts of the history of chromatography dating to about 1903 have been adequately covered by Lederer and Lederer (1), by Consden, Gordon, and Martin (2), and others.

Many techniques and adaptations of methods have widely increased the scope of chromatography. The use of ultraviolet light, suggested by the Portuguese scientist Konig in 1935 (3) to help detect or locate fluorescent substances, has been of great value. In a recent study by Kokoski, *et al.* (4), 133 powdered crude drugs were examined under ultraviolet light and the color of the fluorescence proposed for identification. A disadvantage noted was that there was some variation in color reported for the same crude drug by different observers.

Work supported jointly by the Vanilla Bean Association and the Flavoring Extract Manufacturers Association resulted in the development of a paper chromatographic method for the evaluation of vanilla extracts and the detection of foreign materials used for adulteration (5). An important and interesting result of this work has also been the development of a method whereby fluorescent chromatograms can be photographed in their true colors (6).

At present, the N.F. XI utilizes paper chromatography in the chemical identification test appearing in the monograph on *Rauwolfia serpentina* (7). The alkaloidal content of the sample is compared to a reference standard.

The problem of positive identification of botanical drug extracts is enormous in view of the large variety of botanical species as well as factors affecting the biosynthesis of plant constituents. This is particularly true for those drugs which do not have a pharmacologically active ingredient or chemical component which can be assayed for in a routine manner.

With all of these problems in mind, a rapid method is proposed which uses an ascending paper chromatographic technique with slight modification, ultraviolet light for observation, and, in some cases, the use of appropriate chromogenic spray reagents. This procedure allows for the examination of the drug extract as a whole. The amount of the extract used represents a very small amount of whole crude drug so that identification can be made with only a limited amount of material, the same amount of which may prove difficult or impossible by

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microscopic examination. The resulting chromatogram will produce a characteristic picture and, when compared to a standard or control sample, can then be used for identification.

EXPERIMENTAL

Materials.—Fluid extracts (1 ml. = 1 Gm. crude drug). Tinctures must be concentrated from 0.1–0.2:1 strength to 1:1 ratio. Powdered extracts are reconstituted with the appropriate hydroalcoholic menstruum and filtered or centrifuged to remove insoluble solids (starch, marc, etc.). Solid or pilular extracts are appropriately reconstituted with menstruum to a 1:1 ratio.

Apparatus and Reagents.—A chromatographic chamber whose size will vary with the number of strips run; this may be a box constructed from cardboard, wood, or glass with a removable front and no top; this chamber helps to maintain an atmosphere somewhat saturated with the solvent while protecting the strips from air currents. Crystallizing dishes, 70 mm. in diameter and 50 mm. in depth. Graduated cylinders. Pipet to deliver 0.02 ml. Metal or glass rods and framework to support paper strips. Ventilating hood or similar exhaust system. Ultraviolet lamp with filter passing only long rays (3600–3700 Å.). Filter paper, Whatman No. 4, chromatographic grade, strips cut 2 × 11 in. with the paper fibers running lengthwise. Solvents: *n*-butyl alcohol, glacial acetic acid, distilled water (4:1:5). Chromogenic reagents to be sprayed on the developed and dried chromatographic strip.

Filter Paper Chromatographic Strip.—The paper strip is developed by ascending chromatography. Normal development time, useful for routine identification, requires 2 hours. Longer periods of time did not give improved resolution. Spots A and B represent points of application of the extract. Spot B is a reference spot and is useful to compare the concentration of one extract with another. After development of spot A, the chromatographic picture consists of the base, stem, cone, and solvent front regions. An area above the solvent front region sometimes becomes visible after the chromatogram is treated with an appropriate chromogenic reagent. A drawing representing a typical developed paper strip is shown in Fig. 1.

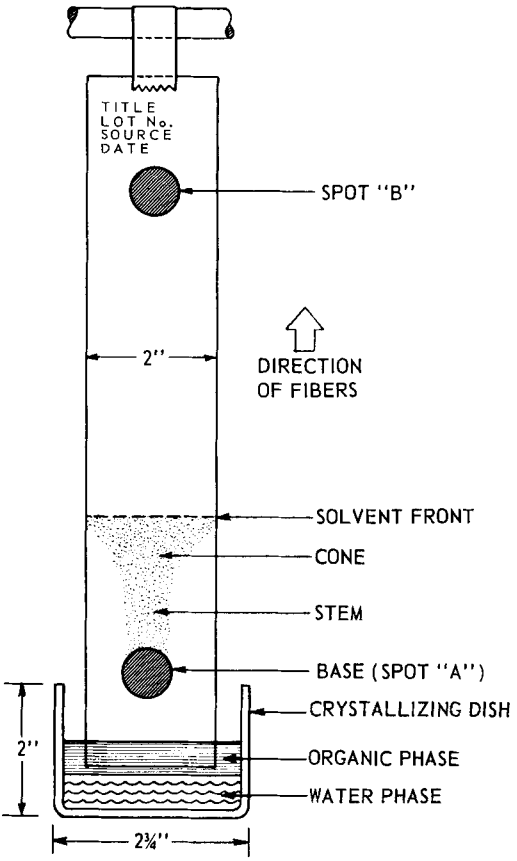

Fig. 1.—A drawing representing a typical developed paper strip.

Procedure.—Prepare the filter paper strip with the title of the extract, lot number, source, date, etc. With a pin and using a guide, mark the exact spots to apply the extract. Spot A centered 1 in. from the bottom edge of the strip and spot B centered 2 in. from the top edge of the strip. Place exactly 0.02 ml. of the extract on spot A and B and allow to dry, about 5 minutes. The extract should be applied slowly to keep the spots as small as possible. Use fluid extract strength (1 ml. = 1 Gm. crude drug). Prepare the solvent consisting of *n*-butyl alcohol, glacial acetic acid, distilled water (40:10:50), and shake vigorously in a separator (stopcock free from grease) for at least 1 minute to saturate before transferring the entire mixture to a crystallizing dish. The solvent is ready to use when the two layers are clear. The solvents should be freshly prepared and of the highest chromatographic grade. Immerse the paper strip in the organic phase so that the bottom edge of the strip is 2–3 mm. above the aqueous layer. Fasten the strips with scotch tape or clamps to a support rod about 12 in. above the bench top. Keep the temperature as constant as possible in the range of 20–25°. For checking reproducibility and comparison, run a reference standard or control and the unknown simultaneously. Each extract should be run in triplicate, not only to gain experience and to confirm reproducibility within each set, but to have additional strips for reagent treatment. Allow the chromatograms to develop for 2 hours with controllable ventilation. It is important to prevent drafts over the paper. Figure 2 shows three sets of chromatograms at the end of a 2-hour developing period. Dry the developed strips in a ventilated hood for 1 hour. The chromatograms should be viewed and evaluated soon after drying because some colors may change or disappear.

Results.—The developed and dried chromatograms are first viewed under visual and then ultraviolet light and the samples compared with the control or standard. Spot B should not show a variation of more than approximately ±10% in intensity. If the intensity varies greatly between the sample and the control, then the extract in question should be either diluted or concentrated to match more nearly the reference standard for identification purposes. Differences in intensity of spot B provide further information in the evaluation

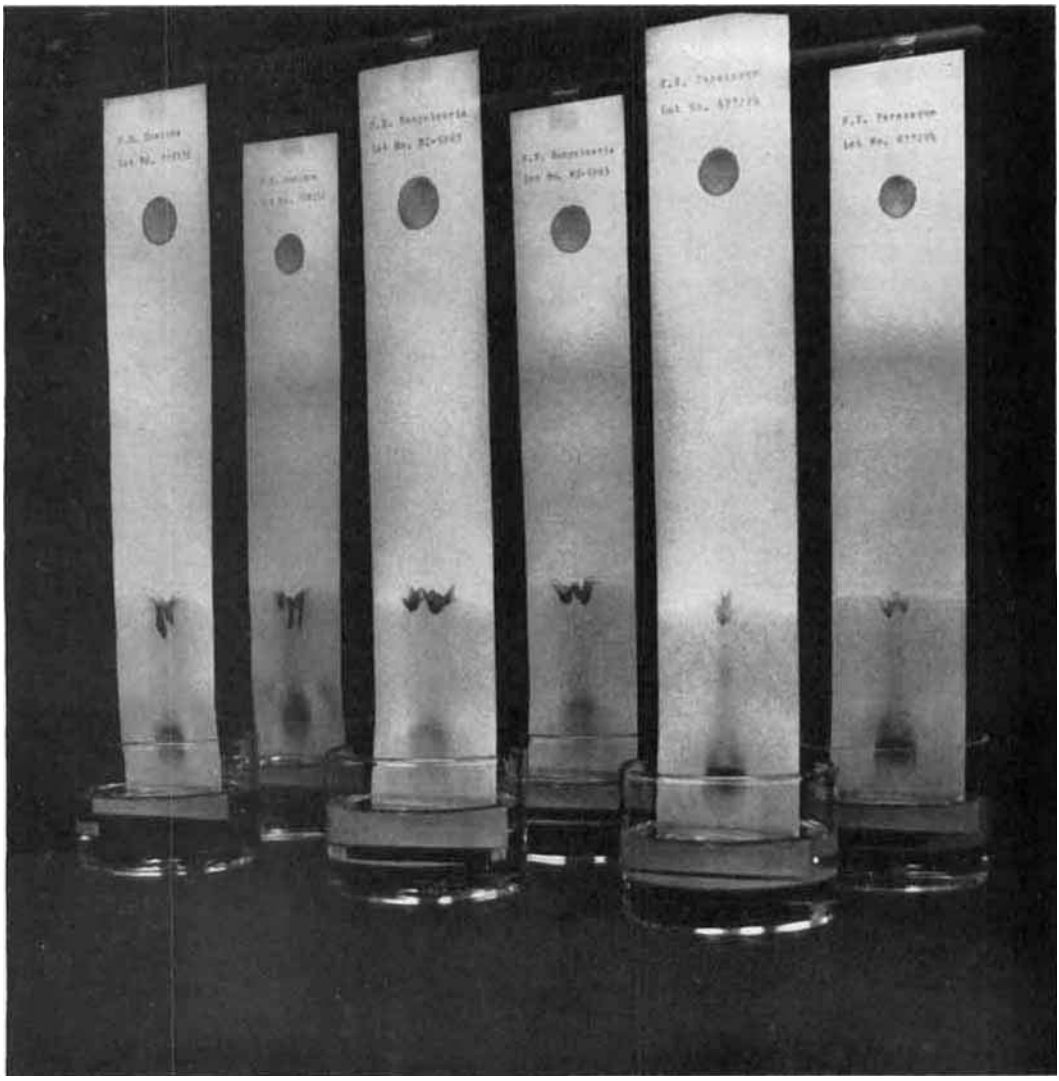

Fig. 2.—Three sets of chromatograms at the end of a 2-hour developing period. For this photograph, the protective side has been removed.

of the sample. A spot of lesser intensity generally indicates a lower concentration of soluble solids in the extract. Spot A has now traveled to form the base, stem, cone, and solvent front regions. Color comparisons should be made of these areas. Shade and intensity should be noted both under visible and ultraviolet light. The degree of travel, distribution, fluorescence, and relative position of the solute must also be observed.

Results have shown that a characteristic picture is produced for each extract tested (Fig. 2). A sample will therefore be positively identified when its developed chromatogram corresponds to that of the control after it has been subjected to the requirements described in the above procedure.

SUMMARY

This technique has proved to be a rapid analytical tool in the routine control, identification, and evaluation of botanical extracts. It produces a chromatogram consisting of base, stem, cone, and solvent front regions. By observing the chromatographic picture as a whole, an unknown can be determined when it matches a control or known sample by producing a total effect similar in distribution, intensity, coloring, and fluorescence. It must be remembered, however, that the variation in details may be due to the natural variation of botanical constituents.

If similar results are obtained with original chromatograms of extracts of different botanicals, further treatment with a chromogenic reagent may provide distinguishing characteristics. Preliminary work has indicated that it is difficult to ascertain the best chemical for use, especially due to the variety of compounds occurring in a

single extract. Effective use has been made of several reagents, however, such as: (*a*) a 0.1 *M* solution of cerium sulfate in water, (*b*) saturated methanol solution of aluminum sulfate, and (*c*) saturated water solution of sodium borate.

The common alkaloidal, sugar, and protein color producing reagents may also be employed. Work is in progress on the tabulation of extracts with corresponding spray reagents and will be described in a subsequent communication.

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Effects of Buffered and Unbuffered Acetylsalicylic Acid upon the Gastric Acidity of Normal Human Subjects

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Acetylsalicylic acid in tablet form, in buffered tablets, and effervescent buffered solution, has been compared with regard to the effect upon gastric acidity and pH. Aspirin and buffered aspirin have little or no effect as compared to controls, whereas the effervescent preparation reduces free acidity and raises gastric pH significantly for 30 minutes. This elevation of gastric pH correlates well with the more rapid absorption and reduced gastric irritation which has been reported for various soluble and buffered forms of salicylates.

DISADVANTAGES of acetylsalicylic acid as an analgetic and antipyretic agent are low solubility, which delays absorption (1), and gastric irritation and bleeding which may result from the erosive action of the crystals of the drug (2), or possibly from its acidity (3), as well as a neurohumoral action (4). It has been shown by Alvarez and Summerskill (5) that there is a causal relationship between salicylate consumption and massive gastrointestinal hemorrhage associated with peptic ulcer. For these reasons, there are a number of proprietary preparations of acetylsalicylate available which are buffered so as to minimize these problems. Several investigators have noted that such buffered preparations are more rapidly absorbed (6, 7). There are also scattered reports that soluble preparations of acetylsalicylic acid or salicylic acid are less irritating and less prone to produce gastric hemorrhage (8, 9). The present investigations were designed to compare the effects of such buffered preparations upon free and total gastric acidity and gastric pH, as compared to unbuffered aspirin.

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† Deceased October 1960.

PROCEDURE

For the initial experiments, subjects were selected at random from available laboratory personnel and included six male and one female subject. They were fasted for 12 hours prior to the experiment and then given a test meal consisting of 50 ml. of cold 45% alcohol, to stimulate gastric secretion. Fifteen minutes after the test meal each subject swallowed a stomach tube, and 5 minutes later withdrew a zero-hour sample of 10 ml. of fluid. Immediately after removal of this control sample the drugs were administered. Over a period of 2 weeks each subject received each drug and performed one experiment where only water was administered (control). Effervescent aspirin[1] was administered dissolved in water, two tablets in 250 ml. of water; buffered aspirin[2] and aspirin[3] were taken whole, two tablets followed by 250 ml. of water. All doses were equivalent to 650 mg. of acetylsalicylic acid. Controls received 250 ml. of water only.

Ten-milliliter samples of gastric juice were removed 10, 20, 30, and 40 minutes after administration of the drugs. All samples were tested for pH using pHydrion paper; for free acid by titration with 0.01 *N* sodium hydroxide to a change in color with Töpfers reagent (pH 3.5); and for total acidity by titration to the end point of phenolphthalein (pH 8.4). Free and total acidity are reported in clinical units (milliliters of 0.1 *N* sodium hydroxide required to neutralize 100 ml. of gastric juice).

[1] Marketed as Alka-Seltzer by Miles Laboratories.
[2] Marketed as Bufferin by Bristol-Myers Co.
[3] Bayer Aspirin.