Extraction of Ergot Alkaloids by Tetrahydrofuran and Its Mixtures*

By J. M. CAMPO† and L. G. GRAMLING‡

The results of a study of the extraction of ergot alkaloids from the crude drug by tetrahydrofuran, tetrahydrofuran-water azeotrope (4.3 per cent water), and tetrahydrofuran-water solution (50 per cent v/v) is presented. The preparation of a tetrahydrofuran-chloroform azeotrope is reported and its composition determined by index of refraction measurements. This azeotrope yielded the highest degree of alkaloidal extraction of any of the tetrahydrofuran mixtures employed. The selectivity of these solvent mixtures for color principles and emulsion-forming constituents of the crude drug is also discussed. All original extractions were carried out in the absence of alkalinity. Thus the ability of the solvents to extract the alkaloids as they occur naturally could be determined.

CARKHUFF (1), in an investigation of various furans as solvents for the extraction of belladonna root, reported the most favorable selectivity for l-hyocyamine by Soxhlet extraction of the ground drug with tetrahydrofuran-water azeotrope. These findings, together with the reported results that tetrahydrofuran in combination with water shows a greater selectivity for caffeine than either solvent alone (2), suggested a further investigation of tetrahydrofuran mixtures for drug extraction.

It was decided to investigate the selectivity of this solvent in the extraction of ergot alkaloids from the crude drug. Whole Spanish ergot, purchased from a reputable distributor, was reduced to a No. 60 fineness and then defatted with petroleum ether. All extractions in this investigation were made from this prepared lot. The tetrahydrofuran which is available commercially contains a stabilizer for the purpose of preventing the formation of peroxides in the solvent (2). The solvent was distilled just prior to extraction.

EXPERIMENTAL

Standardization of the Crude Drug.—Accurately weighed samples (10 Gm.) of the prepared ergot were assayed by the N. F. IX colorimetric method for the determination of total alkaloids (3). The procedure followed was identical to that of the N. F. with the exception of the final dilution of the acid extracts. In this procedure, rather than making one dilution representing 1 Gm. of crude drug per 100 cc. solution, a series of solutions of increasing concentrations, representing from 1 to 5 Gm. of crude drug per 100 cc. solution were prepared. The dilutions were modified in this fashion so that a curve could be established for comparison with the curves formed by similar dilutions of the extracts obtained by the use of tetrahydrofuran and its mixtures.

An ergonovine maleate calibration curve was prepared by making a series of solutions of U. S. P. Ergonovine Maleate Reference Standard in 0.10% tartaric acid solution. Aliquot portions of each solution were taken and allowed to react for thirty minutes with *p*-dimethylaminobenzaldehyde T. S. (3). The color intensities of these solutions were measured in a Klett-Summerson photoelectric colorimeter housing a No. 56 (green) filter (Fig. 1).

The colorimetric readings obtained from the assay of the crude drug by the N. F. IX method for the determination of total alkaloids was plotted on a

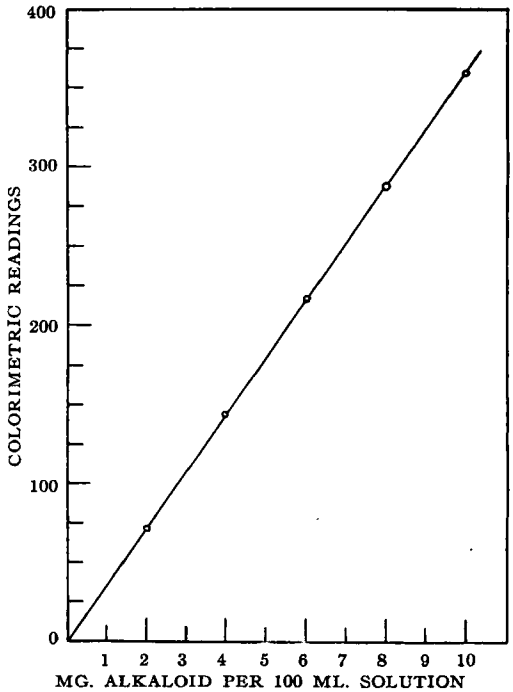

Fig. 1.—Ergonovine maleate calibration curve.

* Received July 25, 1953, from the College of Pharmacy, University of Florida, Gainesville.
Presented to the Scientific Section, A. PH. A., Salt Lake City meeting, August, 1953.
Abstracted from a thesis presented to the Graduate Council of the University of Florida by Joseph M. Campo in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.
† Graduate Fellow, University of Florida.
‡ Associate Professor of Pharmaceutical Chemistry, University of Florida.

graph (Fig. 3). When the reading of the 5-Gm. aliquot portion was referred to the reference curve, it was observed that the crude drug had an alkaloidal content of 7.8 mg. per 5-Gm. sample or 15.6 mg. per total 10-Gm. sample. This expression of total alkaloidal content and those of other assays in this paper are calculated in terms of Ergonovine Maleate Reference Standard.

Extraction with Tetrahydrofuran by Maceration.—Accurately weighed samples (10 Gm.) were macerated with 50 cc. of tetrahydrofuran for a period of forty-eight hours. At the end of the maceration period the supernatant tetrahydrofuran was decanted and the marc washed with a few small portions of fresh tetrahydrofuran to remove residual extract from the marc.

Tetrahydrofuran is miscible with water (2); consequently the extract cannot be applied to partition extraction with sulfuric acid solution as in the case of the official method in which chloroform and sulfuric acid solution are used. To overcome this difficulty, the tetrahydrofuran extract was evaporated to dryness by the use of vacuum without heat, applying the same procedure recommended in the official assay for the concentration of the chloroformic extract (3). The residue remaining upon evaporation of the tetrahydrofuran extract was then dissolved in 30 cc. of a mixture composed of nine parts of chloroform and one part of methanol containing 10% of stronger ammonia T. S. This solvent is identical with that recommended in the official procedure for the original extraction. Only by dissolving the residue in the same solvent as that of the official procedure could a comparable assay be established. The 30-cc. of extract thus obtained was treated in the same manner as in the official procedure with the exception of the final dilutions of the acid extracts which were modified in the same manner as in standardization of the crude drug. Reaction of these solutions with *p*-dimethylaminobenzaldehyde T. S. gave purple indole color reactions, indicating that alkaloids were extracted with nonalkaline tetrahydrofuran. The color intensities of these solutions (Fig. 3) when referred to the reference curve showed an alkaloidal extraction of 9.0 mg. per 10-Gm. sample or 57.8% extraction of total alkaloids.

Soxhlet Extraction.—Accurately weighed samples (10 Gm.) of the prepared ergot were macerated in a soxhlet extractor for one hour with enough tetrahydrofuran to moisten the drug. The sample was then extracted with 50 cc. of tetrahydrofuran adjusting the extraction to one cycle every ten minutes. Extraction was continued for a period of four hours. Assay of the extract showed an alkaloidal extraction of 9.4 mg. per 10-Gm. sample or 60.3% extraction of total alkaloids (Fig. 3).

Extraction with Tetrahydrofuran-Water Azeotrope (2) by Maceration.—Accurately weighed samples (10 Gm.) of the prepared ergot were macerated for forty-eight hours with 50 cc. of tetrahydrofuran-water azeotrope. Assay of the extract showed an alkaloidal extraction of 12.4 mg. per 10-Gm. sample or 79.4% extraction of total alkaloids (Fig. 3).

Soxhlet Extraction.—Accurately weighed samples (10 Gm.) of the prepared ergot were macerated in a Soxhlet extractor for one hour with enough tetrahydrofuran-water azeotrope to moisten the drug. The sample was then extracted with 50 cc. tetrahydrofuran-water azeotrope for a period of four hours. Total alkaloidal assay of this extract showed an alkaloidal extraction of 12.0 mg. per 10-Gm. sample or 76.9% extraction of total alkaloids (Fig. 3).

It was observed that the extracts obtained by both maceration and Soxhlet extraction with tetrahydrofuran-water azeotrope possessed a slight red color which was not observed in the extraction with tetrahydrofuran. This indicated extraction of the water-soluble color principles of the crude drug by the water present in the azeotrope. In both cases, however, the color was satisfactorily removed during the alkaloidal purification procedures of the assay.

Extraction with Tetrahydrofuran-Water Solution (50% V/V).—As noticed in the previous determinations, the presence of water in azeotropic combination with tetrahydrofuran has served to potentiate the alkaloidal extraction properties of the solvent. With this in mind, it was decided to extract the ergot with a tetrahydrofuran-water solution containing equal volumes of both solvents.

It is obvious that Soxhlet extraction cannot be used to measure the degree of alkaloidal extraction by using a 50% tetrahydrofuran-water solution, as the solvent which comes in contact with the crude drug would be the standard tetrahydrofuran-water azeotrope having a water composition of 4.3%. It was decided, therefore, that extraction of the ergot with this 50% solution must be restricted to maceration.

Accurately weighed samples (10 Gm.) of the prepared ergot were macerated with 50 cc. of a solution containing equal parts of tetrahydrofuran and water. The length of time of maceration was forty-eight hours as in the other determinations. The extract thus obtained had a deep red color, the intensity being much greater than that of the tetrahydrofuran-water azeotrope extract. This observation further helped to establish increased selectivity for

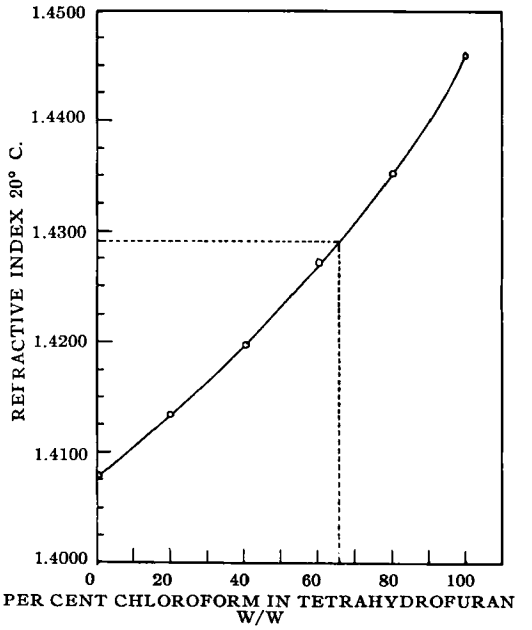

Fig. 2.—Refractive index of tetrahydrofuran-chloroform mixtures.

the color principles of the crude drug with increasing water content of the solvent. The color was removed during the purification procedures of the assay, which showed an alkaloidal extraction of 7.8 mg. per 10-Gm. sample or 50% extraction of total alkaloids (Fig. 3).

Preparation and Determination of Composition of Tetrahydrofuran-Chloroform Azeotrope.—This azeotrope was prepared by distilling a mixture of 200 cc. of tetrahydrofuran and 200 cc. of chloroform. The temperature gradually rose to 72.5° and remained at that temperature during the major course of the distillation. Only the constant boiling azeotrope (72.5°) was collected.

In order to determine the composition of the tetrahydrofuran-chloroform azeotrope a series of weight to weight solutions of chloroform in tetrahydrofuran were prepared and the refractive index of each solution determined by means of an Abbe refractometer at 20°. Using these data a refractive index curve was plotted (Fig. 2). The refractive index of the tetrahydrofuran-chloroform azeotrope taken at 20° was found to be 1.4292. Referring this reading to the refractive index curve revealed that the composition of the azeotrope was 65.5% chloroform in tetrahydrofuran (w/w).

Extraction with Tetrahydrofuran-Chloroform Azeotrope by Maceration.—Before considering the extraction procedure with the tetrahydrofuran-chloroform azeotrope, it would be best to point out first that this combination was found to be immiscible with water. It will be remembered that all combinations used up to this point have been miscible with water and consequently the necessary modification of the official procedure was necessary before these solvents could be applied to partition extraction. Complete evaporation to dryness is not necessary with this azeotrope because of its immiscibility with water, and the N. F. procedure may be more accurately employed.

Accurately weighed samples (10 Gm.) were extracted by the maceration procedure previously employed. Assay of the extracts showed an alkaloidal extraction of 14.4 mg. per 10-Gm. sample. The extent of alkaloidal extraction was thus 92.3% of total alkaloids (Fig. 3).

Soxhlet Extraction.—Assay of the extracts obtained by Soxhlet extraction of accurately weighed samples (10 Gm.) showed an alkaloidal extraction of 13.8 mg. per 10-Gm. sample or 88.5% extraction of total alkaloids (Fig. 3).

The extracts obtained by both maceration and Soxhlet extraction of the crude drug with tetrahydrofuran-chloroform azeotrope were very light in color, indicating a minimum of selectivity for the color principles of the crude drug. It was further observed that this azeotrope did not form emulsions as readily as with the other procedures employed during assay, indicating that the selectivity of this solvent for emulsion-forming constituents of the crude drug was very low.

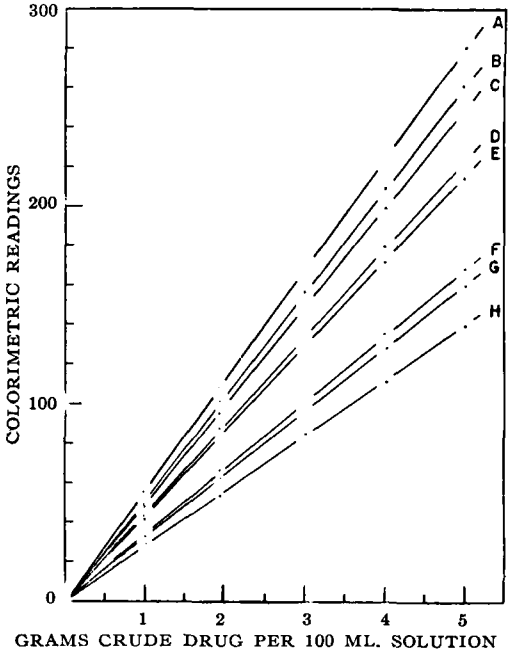

Fig. 3.—Extraction of ergot alkaloids.

A. N. F. IX assay. *B*. Extraction with tetrahydrofuran-chloroform (maceration), *C*. Extraction with tetrahydrofuran-chloroform (Soxhlet). *D*. Extraction with tetrahydrofuran-water (maceration). *E*. Extraction with tetrahydrofuran-water (Soxhlet). *F*. Extraction with tetrahydrofuran (Soxhlet). *G*. Extraction with tetrahydrofuran (maceration). *H*. Extraction with tetrahydrofuran-water 50/50 (maceration).

Assay of the Marcs for Residual Alkaloids.—The investigation up to this point has shown that each of the various solvent combinations employed has resulted in a specific degree of alkaloidal extraction. It became necessary therefore to determine whether this difference in the extent of alkaloidal extraction was due to selectivity for specific alkaloids or whether alkaloidal destruction was responsible for the

TABLE I.—SUMMARY OF RESULTS OBTAINED FROM EXTRACTION OF CRUDE ERGOT DRUG BY VARIOUS SOLVENTS

Mg. Alkaloid per 10-Gm. Sample	Solvent	Procedure	Mg. Alkaloids Extracted	Extraction, %
15.6	THF[a]	Maceration	9.0	57.8
15.6	THF	Soxhlet	9.4	60.3
15.6	THF-water azeotrope	Maceration	12.4	79.4
15.6	THF-water azeotrope	Soxhlet	12.0	76.9
15.6	THF-water (50% v/v)	Maceration	7.8	50.0
15.6	THF-$CHCl_3$ azeotrope	Maceration	14.4	92.3
15.6	THF-$CHCl_3$ azeotrope	Soxhlet	13.8	88.5

[a] Tetrahydrofuran.

difference. The marcs which were obtained by the various methods of extraction were dried and saved for this phase of the investigation. Each marc was assayed by the N. F. IX method or the determination of total alkaloids (3), to determine the presence of any alkaloid which might not have been extracted by the tetrahydrofuran mixtures. Very faint indole color reactions were obtained only from the marcs which were previously extracted with tetrahydrofuran alone and with tetrahydrofuran-chloroform azeotrope. Colorimetric determinations indicated that in each of these cases only 1 mg. of total alkaloids was found. Extracts obtained from the remaining marcs gave negative indole color reactions indicating that there were no alkaloids in these marcs. The data suggest that the differences in the degree of alkaloidal extraction is not due to specific selectivity, but rather to varying degrees of alkaloidal destruction.

Table I represents a summary of the data obtained by extraction of the crude drug by the various solvent combinations employed.

REFERENCES

(1) Carkhuff, E. D., and Gramling, L. G., THIS JOURNAL 41, 660(1952).
(2) "Tetrahydrofuran," E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.
(3) "The National Formulary," 9th ed., Mack Publishing Co., Easton, Pa., 1951, p. 191.

Further Studies on the Metabolic Disposition of Dormison (3-Methyl-pentyne-ol-3) in Dogs and Man*

By PRESTON L. PERLMAN, DAVID SUTTER, and CAROL B. JOHNSON

A modified method for the determination of Dormison in biological materials is reported. The metabolic disposition of Dormison in dogs and in man was studied and the results of this investigation are presented.

IN A PREVIOUS publication (1) a series of animal experiments were described which had as their purpose the study of the modes of excretion and metabolic fate of the hypnotic carbinol, Dormison.[1] These experiments indicated that a major step in the metabolism of Dormison is the destruction of the ethinyl group.

Additional studies, in these laboratories, to extend the body of information on the metabolic disposition of Dormison have been carried out in dogs and in man and the results are the subject of this report.

The possible elimination of the drug as a conjugate was considered. Dormison, a tertiary alcohol, might be expected to conjugate with glucuronic acid. In addition, the role of the lungs and gastrointestinal tract as eliminative pathways have been examined.

* Received May 4, 1953, from the Biological Laboratories, Schering Corporation, Bloomfield, N. J.
[1] Registered trademark of Schering Corporation, Bloomfield, N. J.

METHODS

Dormison.—It was observed that the original method of analysis (1) yielded, on occasion, unaccountable high Dormison values when certain urine samples were analyzed. These elevated values occurred about the third to fourth day following drug administration, and appeared to be associated with the presence of an oily, black sediment which formed when ether extracts of the urine were reacted with the alkaline silver reagent. The sediment proved to be metallic silver which suggested the presence of an increased amount of reducing substance (*s*) in the urine. To circumvent the difficulty of analysis, a modification of the analytical procedure was introduced which embodied the basic principle of the original method, i. e., the reaction of the triple bond with 2 moles of silver. At the same time, the modification overcame the presence of interfering substances by using an aeration system for distilling the Dormison from the sample under analysis. The method as finally evolved follows.

An aliquot (75 ml.) of a twenty-four hour urine specimen or a fecal slurry sample is placed in a 250-ml. Pyrex centrifuge bottle fitted with a two-hole stopper and glass tubing as illustrated in Fig. 1. The bottle is placed in a hot water bath (80–85°) and aeration begun into a centrifuge bottle containing the alkaline silver reagent using a water aspirator system. A dilute sulfuric acid trap is installed between the alkaline silver reagent bottle and the aspirator to prevent escape of ammonia vapors into the laboratory. It was noted that the aeration would