The Quantitative Separation of Ergometrine from Other Ergot Alkaloids*

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Since the discovery of the water-soluble alkaloid, ergometrine (ergonovine), and the recognition of its predominate therapeutic importance, the need of a quantitative method for its determination in ergot and ergot preparations has been imperative. The alkaloids of ergot occur in isomeric pairs, five such pairs having been isolated and identified to date. Only one isomer of each of these pairs is physiologically active, and only one pair, ergometrine-ergometrinine, is water-soluble, the ergometrine representing in this case the physiologically active component. When ergot or an ergot preparation is taken orally, the greater portion of the physiological response is probably due to the water-soluble alkaloid, ergometrine.

The separation of the water-soluble alkaloids from those which are water-insoluble has already been accomplished, Hampshire and Page (1), Trabucchi (2), Allport and Porter (3), Schou and Tonnesen (4), Casparis and Bullet (5) and Schumacher (6). A survey of these methods revealed that none of them was adequate for the quantitative separation of ergometrine from its inactive isomer, ergometrinine. It was evident, therefore, that the present investigation should start with an attempt to separate the two water-soluble alkaloids.

The ergotoxine ethanesulfonate, ergotinine, ergosine, ergosinine, ergometrine and ergometrinine which were used for the experimental work were purchased from Burroughs Wellcome & Co. Ergotamine tartrate was obtained from Sandoz Chemical Works, Inc. A very small sample of ergometrine, prepared in this laboratory from ergot and purified by many recrystallizations from chloroform, was used as a standard for comparing the purchased ergometrine. The optical crystallographic properties of both samples of ergometrine were found to be identical. The ergometrinine had $[\alpha]_D^{20}$ + 423° in chloroform (c = 0.1). Smith and Timmis (8) reported a value of +414° for this alkaloid. Because of the small quantities available, all of the above alkaloids were used as purchased without any further attempt at purification.

Allport and Porter (3) made use of an antimony trichloride reagent which precipitated ergometrine, but not ergotoxine. While this work was confirmed, studies with ergometrinine showed that this alkaloid was also precipitated almost quantitatively by this reagent and, therefore, the method would be of no value in the separation of ergometrine from ergometrinine. The procedure of Trabucchi (2) using picric acid was tried and it was found that while ergotoxine was precipitated, ergometrine and ergometrinine remained together in solution. Morrell and Chapman (private communication) have been able to separate the water-insoluble alkaloids from ergometrine by means of a phosphomolybdic-phosphotungstic acid reagent. They had no ergometrinine available to test, but were of the opinion that it probably would remain in solution with the ergometrine. This has been confirmed and, therefore, their reagent would not effect a separation of these two alkaloids.

It was evident from the above that the possibility of finding a reagent that would precipitate one of these isomeric alkaloids, and not the other, was not very promising, so this method of attack was abandoned. The manner in which these two alkaloids distributed themselves between water and various immiscible solvents was studied in the hope of finding a solvent that would effect a separation. The procedure used was as follows:

EXPERIMENTAL

Varying amounts of the alkaloid were dissolved in slightly ammoniacal water (pH = 10), these water solutions were analyzed colorimetrically, using the reagent of Allport and Cocking (7) and a Brice (9) abridged Photoelectric Spectrophotometer (filter for 560 mμ). Aliquots were taken and shaken for 10 minutes with equal volumes of the immiscible

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solvents at room temperature (approx. 25° C.). The water layer was then withdrawn and the amount of alkaloid remaining in the water determined colorimetrically. The results obtained are given in Table I.

Table I.—Distribution of Ergometrine and Ergometrinine between Equal Volumes of Water and Various Immiscible Solvents

Immiscible Solvent	Per Cent Remaining in Water: Ergometrine	Ergometrinine
Ether (U. S. P.)	88.3	28
Dichlorethylene	43.7	3.4
Chloroform	26.0	3.4
Trichlorethylene	90.3	29
Isopropyl ether	93.4	43
Carbon tetrachloride	100	85
Benzene	81.6	32
Methylene chloride	52.4	23
Ether, 2 vols. Dichlorethylene, 1 vol.	49	7.6

Of all the solvents tried, ether seemed to offer the most promise in that it causes less emulsification when used with the drug, exists as the top layer in the separator and allows as great a separation of the two alkaloids as any of the other solvents. A consideration of the distribution ratio of ergometrine and ergometrinine between ether and water disclosed that if an ether solution of them was shaken repeatedly with water, all of the ergometrine would be extracted together with considerable ergometrinine. However, if the water extracts were washed successively through a second and a third separator containing ether, most of the ergometrinine would be eliminated. Using the distribution ratio of these alkaloids between water and ether, theoretical calculations were made to determine, using three ether-containing separators, the proper volume ratios of the solvents and the optimum number of water extractions to be made to give the most complete separation. The method finally adopted was as follows:

Twenty-five cc. of a 1% tartaric acid solution of the alkaloids was placed in a separator and made faintly alkaline to litmus with ammonia, 35 cc. of U. S. P. ether was added and the mixture shaken for three minutes by hand. It is essential that this period of shaking be used in making all of the extractions throughout this procedure. The separator was then inverted and the stop-cock cautiously opened to permit the release of excess pressure. The two layers were allowed to separate sharply, and the water layer was drawn off as completely as possible into a second separator containing 35 cc. of ether. The second separator was shaken and the water withdrawn into a third separator containing 35 cc. of ether. After shaking in the third separator, the water was drawn off into a 250-cc. beaker. The whole process described above was repeated with six successive 10-cc. portions of weak ammonia water (prepared by adding one drop of 10% NH_3 W/V to 200 cc. of water, giving a pH of approximately 10), and the water extracts were combined in the 250-cc. beaker. The dissolved ether was removed from the combined water extracts by warming slightly on the steam bath (40–45°) while blowing a current of air over the water, the solution cooled, made to 100 cc. and the alkaloids determined colorimetrically as previously described. The volume ratios of water and ether should be strictly adhered to, as any change in these ratios will affect the percentage distribution of the alkaloids.

One per cent tartaric acid solutions of the various alkaloids were prepared, assayed colorimetrically, and 25-cc. aliquots were run through the above-described procedure. The results obtained when ergometrine and ergometrinine were run separately and when mixed together are shown in Table II, as well as results for a mixture of the five water-insoluble alkaloids and mixtures of all seven of the alkaloids available for this study.

Table II.—Determination of Ergometrine in Various Mixtures

Alkaloid	Amount Taken, Mg.	Recovered in Water Ext. Calc. as Ergometrine: Mg.	%
Ergometrine	2.092	2.077	99.3
	1.520	1.494	98.3
Ergometrinine	2.442	0.135	5.5
	2.230	0.107	4.8
Ergometrine +	0.831 + 0.977	0.881	106
Ergometrinine	0.912 + 0.446	0.921	101
Water-insoluble alkaloids[a]	10.943	0	0
Water-insoluble	6.566 + 1.050 + 0.466	1.105	105
alkaloids +	5.926 + 1.009 + 0.504	1.009	100
Ergometrine + ergometrinine	6.914 + 0.504 + 0.504	0.531	105

[a] The water-insoluble alkaloids consisted of approximately equal parts of ergotoxine, ergotinine, ergotamine, ergosine and ergosinine. The figure given is for total alkaloids.

It can be seen from the results in Table II that about 98% to 99% of ergometrine can be recovered and about 5% of the amount of ergometrinine present comes through. This would tend to give slightly high results for ergometrine, the degree depending upon the quantity of ergometrinine present. It was found that in running ergometrinine alone, the last ether wash always contained about 15%

of the amount taken. Therefore, in applying this method to the assay of ergot, the last ether wash can be extracted with 1% tartaric acid and an approximation of the amount of ergometrinine can be obtained. From some preliminary results recently obtained on two different samples of ergot, the ergometrinine present amounted to about half of the ergometrine content. If this is true for all samples of ergot, the error due to ergometrinine would amount to about 2-3%.

None of the five water-insoluble alkaloids tested came through in the water extract. It is regrettable that the remaining three water-insoluble alkaloids, ergotaminine, ergocristine and ergocristinine were not available for testing. It is felt, however, that they will not interfere with the method as they are all water-insoluble, ether-soluble alkaloids.

After the water-insoluble alkaloids (no ergometrine or ergometrinine present) had been run through the ether system, the second and third ether washes were shaken out separately with 1% tartaric acid and the alkaloid determined colorimetrically. It was found that the second ether contained 5.7% of the total amount of alkaloid used at the start of the assay and the third ether contained only approximately 0.2%. This indicates that in use of the Hampshire and Page (1) technique, about 6% of the water-insoluble alkaloids may be present in the water extract. Casparis and Bullet (5) reported as much as 12% of these alkaloids carried over into the water when they used the Hampshire and Page method.

Further work is being done at the present time adapting this separation scheme to the assay of ergot, the results of which will be reported in a future paper. It is hoped that this method will result in an agreement between the chemical and biological assays for ergometrine.

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SUMMARY

A method for the quantitative separation of ergometrine from the water-insoluble ergot alkaloids as well as from its therapeutically inactive isomer, ergometrinine, has been presented.

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A Phytochemical Study of *Aloe vera* Leaf*

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The study reported in this paper was undertaken in connection with an attempt to isolate and characterize the principle contained in *Aloe vera* leaf which is responsible for its activity in promoting the healing of third degree x-ray reactions on white rats. Although dried aloe and its constituents have been the subject of numerous investigations, little work has been done on the fresh aloe leaf. Hence, much of the work reported herein is preliminary in nature. Most of the cursory investigations of the fresh leaf which have appeared from time to time have been of a histological and anatomical nature.

Robiquet, in 1846 (1), reported studies which have since been contradicted. Reports by Unger in 1855 (2), Gasparrini in 1863 (3) and Trecul in 1872 (4) contributed little to our chemical knowledge of the leaf. Prollius in 1884 (5) contributed perhaps the most informative report on several species of aloe, while the latest work of Chopra and Ghosh, in 1938 (6), on fresh *Aloe vera* leaf reported the presence of a trace of volatile oil, non-volatile oil, resin, gum, emodin, an anthraquinone compound, and chrysophanic acid. These constituents, plus aloin, are essentially all that have been found in previous investigations.

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