Fluorometric Assay of Yohimbine

By H. C. CHIANG and W. F. CHEN

The fluorescence of solutions of yohimbine under ultraviolet light is increased by heating with hydrogen peroxide and is quantitatively related to its concentration. An assay procedure has been developed for pharmaceutical preparations containing yohimbine.

YOHIMBINE has been used medicinally as an aphrodisiac, but there is no convincing evidence for such an effect (1). However, in Formosa it is often illegally mixed in male hormone preparations. Consequently, there is an urgent need to find a suitable method for determining yohimbine in such preparations.

Previous analytical methods have been based on the color reactions of yohimbine with Reinecke salt (2), *p*-dimethylaminobenzaldehyde (3), vanillin (4), and xanthydrol (5). But the interference of hormones as well as vitamins make the above methods unsuitable for the determination of yohimbine in these combinations.

Yohimbine and other rauwolfia alkaloids have fluorescence under ultraviolet light, and this property has been used to determine their location on chromatograms (6–8).

We have found that the intensity of the fluorescence of yohimbine solutions heated with hydrogen peroxide is proportional to the concentration. This procedure has proved to be effective in determining yohimbine in preparations containing methyltestosterone, vitamin B_1, vitamin B_2, *dl*-methionine, and strychnine nitrate.

EXPERIMENTAL

Reagent.—Standard yohimbine solution containing 20 mcg. per ml. in 5 *N* acetic acid.

Apparatus.—Kotaki ultramicro fluorophotometer with 48 × 40 × 8 mm. cells.

Reaction.—It was found that a suitable fluorescence could be obtained by adding 1.0 ml. of 3% hydrogen peroxide to the solution and heating the tube in a boiling water bath for about 45 minutes. The fluorescence developed in this manner showed no change on standing 10 hours. Also the concentration of yohimbine to fluorometric reading follows a straight line relationship. The results of these experiments are summarized in Fig. 1.

Determination of Yohimbine in Hormone-Vitamin Preparations.—Transfer a portion of powder equivalent to 10 mg. of yohimbine-HCl to a 100-ml. volumetric flask. Add 2% tartaric acid solution to the mark, and shake the mixture occasionally during 2 hours; filter, if necessary, rejecting the first 20 ml. of filtrate. Pipet 20 ml. of filtrate into a separator. Then add ammonia T. S. to distinct alkalinity, and extract yohimbine with 20, 20, 10, 10, and 10-ml. quantities of ether. Wash the combined ether extracts with two 10-ml. portions of water. Extract the washings with 10 ml. of ether; add this ether to the combined ether extracts and evaporate the ether on a steam bath to dryness. Dissolve the residue in 100 ml. of 5 *N* acetic acid to make a solution of concentration of 20 mcg./ml.

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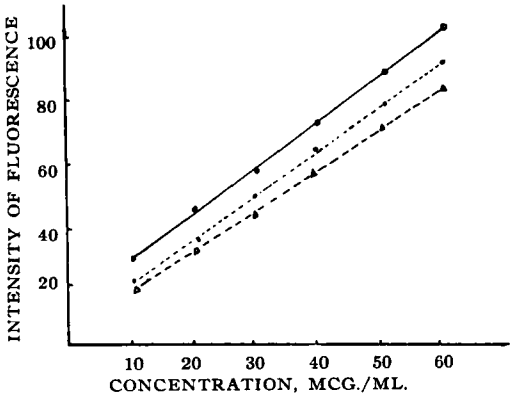


Fig. 1.—Relationship between heating time, concentration, and intensity of fluorescence. Key: O——O——O, 45–60 minutes of heating time; ● . . . . . ● . . . . . ●, 15 minutes of heating time; △ – – – △ – – – △, 5 minutes of heating time.

TABLE I.—SAMPLE PRESCRIPTIONS AND RESULTS OF EXPERIMENTS

Description	Capsule 1, mg.	Capsule 2, mg.	Capsule 3, mg.	Mixture 1, mg.
Methyltestosterone	10	10	10	10
Vitamin B_1	5	10	10	10
Vitamin B_2	2.5	...	...	2.5
dl-Methionine	35	...	...	30
Strychnine nitrate	...	1	...	1
Amino ethane sulfonic acid	...	...	30	30
Calcium glycerophosphate	...	...	200	200
Yohimbine-HCl	2	2	2	0
Av. recovery, %	100.1 ± 1.8	101.6 ± 1.4	99.4 ± 0.2	0

Accurately pipet 2.0 ml. of this solution and standard solution respectively into a 10-ml. volumetric flask. Add 1 ml. of 3% hydrogen peroxide to each, dilute to exact volume with 5 *N* acetic acid, and mix well. Heat in boiling water for 45 minutes. Then cool to room temperature and measure the fluorescence. The concentration of yohimbine hydrochloride in the sample solution is calculated

$$\frac{\text{Galvanometer reading of sample}}{\text{Galvanometer reading of standard}} \times \text{mcg. of yohimbine HCl in standard solution} = \text{mcg. of yohimbine HCl in sample solution.}$$

RESULTS AND DISCUSSION

The proposed method of assay is applied to the mixtures of known yohimbine content. Sample prescriptions and results of experiments are presented in Table I. The results have been shown to be free of interference from most of the vitamins and substances frequently associated with yohimbine.

REFERENCES

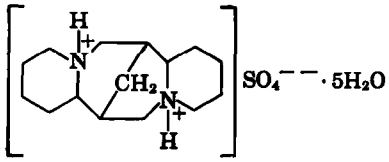
(1) Goodman, L., and Gilman, A., "The Pharmacological Basis of Therapeutics," The Macmillan Co., New York, N. Y., 1956, p. 587.
(2) Staininer, C., and Capiere, C., *J. Pharm. Belg.*, **8**, 3(1954).
(3) Kolsek, J., *Z. Anal. Chem.*, **140**, 186(1953).
(4) Kurada, G., and Tamamura, S., *Yakkyoku*, **7**, 1193 (1956).
(5) Jung, Z., *Cesk. Farm.*, **6**, 299(1957).
(6) Sakal, E. H., and Merrill, E. J., THIS JOURNAL, **43**, 709(1954).
(7) Klohs, M. W., *J. Am. Chem. Soc.*, **76**, 1381(1954).
(8) Schlitter, E., and Schwarz, H., *Helv. Chim. Acta*, **33**, 1463(1950).

Quantitative Determination of Sparteine Sulfate

By PAUL TURI and DAN GROSSMAN

Official compendia fail to include assay methods for pharmaceutical preparations of sparteine sulfate. A direct acidimetric titration method for sparteine sulfate and an indirect titration of the sparteine base, obtained by distillation from a solution of sparteine sulfate, were investigated in a comparative study. The accuracy and applicability of both methods are evaluated.

SPARTEINE, an alkaloid of *Cytisus scoparius* L. (syn. *Sarothamnus scoparius*, Koch), *Lupinus luteus* L. and other plants of the *Leguminosae*, has been known as a medicinal agent for more than 70 years (1).



Sparteine had been formerly recommended in tachycardia, functional palpitation of the heart, and as a diuretic agent.

"The National Formulary," 8th ed., 1946, monograph (2) for sparteine sulfate (the last official U. S. compendium to list this drug) fails to include a method of assay. Recent interest in sparteine sulfate as an adjunct in induction of labor (3) prompted an evaluation of the known quantitative assay procedures.

In a survey of several suggested methods Higuchi and Bodin (4) list four methods for the determination of sparteine sulfate: (*a*) a colorimetric assay through the Reineckate salt, (*b*) an acid dye procedure (assaying colorimetrically a chloroform extract of the compound formed with acid methyl orange), (*c*) a polarographic method, and (*d*) a gravimetric determination through the silicotungstate salt.

The official method in the "Pharmacopoea Helvetica V" (5) for sparteine sulfate assay is a direct acidimetric titration. This procedure is based on the principle that mineral acid salts of very weak nitrogen bases in aqueous solution can be titrated directly with strong alkaline titrants to the phenolphthalein red end point, since the liberated weak base does not interfere with the indicator (6).

Our objective was to select a simple and rapid method with satisfactory accuracy. The direct titration method requires only a single step; therefore its advantages are obvious. To increase the specificity of the analysis, we adapted another titration technique which included a separation step. The principle of distillation of the alkaloid base and back titration of volatile alkaloids was utilized.

The scope of this study is a comparison between the two acidimetric titration methods: *method I*, sparteine sulfate is titrated directly with 0.1 *N* sodium hydroxide solution; and *method II*, sparteine sulfate salt is converted to the base, distilled with steam into a measured volume of acid, and the excess acid is back-titrated.

EXPERIMENTAL

Reagents and Test Solutions

Sparteine sulfate: a commercial sample of sparteine sulfate, N.F. VIII, was used in this study without further purification m.p. 136° dec., $[\alpha]_D^{25} = -21.5°$ (c = 4 in water); 0.1 *N* NaOH; 0.1 *N* HCl; methyl orange T.S.; phenolphthalein T.S.; hydrogen peroxide T.S.; 0.1 *N* H_2SO_4; sulfuric acid, diluted; 0.1 *N* $KMnO_4$; 50% NaOH: 10 Gm. of NaOH (U.S.P. XVI) dissolved in 10 ml. of water. Volumetric and test solutions meet U.S.P. XVI specifications.

Analytical Procedures

Sample A.—Approximately 1.0 Gm. of sparteine sulfate ($C_{15}H_{26}N_2 \cdot H_2SO_4 \cdot 5H_2O$, mol. wt. 422.53), accurately weighed, was dissolved in water in a 25-ml. volumetric flask, and the volume was adjusted to mark at 20° (stock solution).

Method I: 5.0 ml. of the stock solution was transferred into an Erlenmeyer flask, one drop of

TABLE I.—COMPARATIVE ASSAY RESULTS FOR SPARTEINE SULFATE

Sample No.	Sample Type	Method I %	Method I d_a	Method II %	Method II d_a
1	A	100.43	0.13	99.68	−0.60
2	A	100.43	0.13	98.84	−1.44
3	A	100.35	0.05	100.88	0.60
4	B	101.00	0.70	101.50	1.22
5	B	100.00	−0.30	102.50	2.22
6	B	99.00	−1.30	100.00	−0.28
7	B	99.50	−0.80	101.50	1.22
8	B	100.50	0.20	99.00	−1.28
9	B	101.50	1.20	98.60	−1.68

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