THE CHROMATOGRAPHIC PURIFICATION AND ULTRA-VIOLET SPECTROPHOTOMETRIC ESTIMATION OF HYDRASTINE AND BERBERINE IN FLUID EXTRACT OF HYDRASTIS

By M. S. El Ridi, K. Khalifa and A. Mamoon

From the Biochemistry Department, Faculty of Medicine, Kasr El-Aini, Cairo University, Egypt

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Of the three alkaloids known to be present in *Hydrastis canadensis* only hydrastine is used therapeutically, although berberine and canadine have some physiological activity[1]. The B.P.C. 1949 assay procedure for hydrastis has a number of undesirable features. It is a gravimetric procedure and includes small amounts of non-alkaloidal substances not easily removed during purification. The assay is expensive in materials and time. It does not determine hydrastine but the sum of hydrastine and canadine and it does not estimate berberine.

The volumetric methods[2–4] proposed for the assay of berberine in drugs may have certain advantages over the gravimetric procedure but no specific method is available for berberine and hydrastine. Brochmann-Hanssen and Evers[5] described a fluorimetric method for the determination of hydrastine in hydrastis. The fact that berberine occurs in hydrastis in considerable quantities although its pharmacological action is two-fifths that of hydrastine[6] renders necessary a specific method for its estimation. The chemical methods of estimation of berberine are complicated and need the purification of the alkaloid when applied to hydrastis.

This work describes an ultra-violet spectrophotometric method for the identification and estimation of hydrastine and berberine in hydrastis.

Experimental

The ultra-violet absorption spectra of pure samples of hydrastine and berberine hydrochloride have been examined using absolute ethanol as a solvent by the Unicam spectrophotometer. Hydrastine ($C_{21}H_{21}O_6N$, B.P.C. 1949; m.pt. 132° C.) shows a maximum absorption at 297 mμ with E (1 per cent. 1 cm.) 200.

Berberine hydrochloride ($C_{20}H_{18}O_4N,HCl.2H_2O$, B.P.C. 1934) shows two maximas, one at 270 mμ and the other at 350 mμ with E (1 per cent. 1 cm.) 600 [i.e. E (1 per cent. 1 cm.) of pure berberine alkaloid ($C_{20}H_{19}O_5N$) is 694].

The E (1 per cent. 1 cm.) of each alkaloid is plotted against wavelength and the absorption curves are illustrated in Figure 1.

It is clear from Figure 1 that the absorption at 350 mμ is due only to berberine. Therefore in a pure mixture of hydrastine and berberine, only the amount of berberine can be calculated from the extinction measured at 350 mμ. On the other hand, direct spectrophotometric measurement of the extinction due to hydrastine at 297 mμ is difficult

as the absorption due to berberine overlaps that of hydrastine in this region.

For the estimation of hydrastine in hydrastine-berberine mixtures it is necessary therefore to extract hydrastine quantitatively with ether in presence of ammonia. The ether is distilled and the extracted hydrastine is dissolved in absolute ethanol (or 96 per cent.) and adjusted to a known volume. The amount of hydrastine is determined by measuring the extinction at 297 mμ. This spectrophotometric method was compared with the B.P.C. 1949 assay procedure on prepared amounts of hydrastine berberine mixtures. The results are given in Table I.

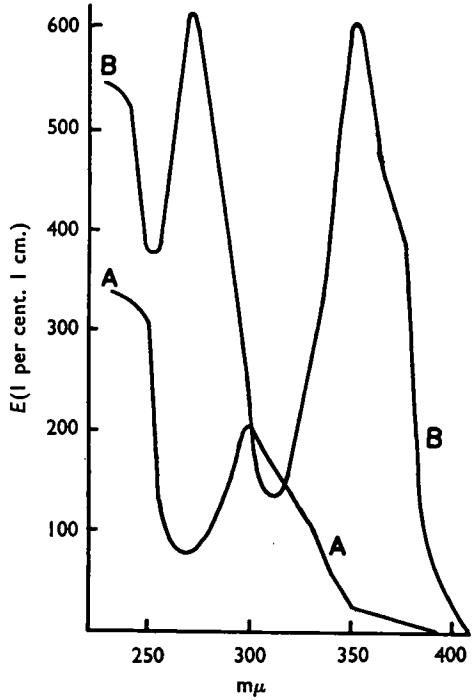

FIG. 1. Showing the absorption of (A) hydrastine and (B) Berberine hydrochloride in absolute ethanol.

Determination of Hydrastine and Berberine in Galenicals

In galenical preparations of hydrastis, berberine always accompanies hydrastine. These alkaloids may be separated by the adsorption chromatographic technique[7,8]. The purified product is then estimated by the spectrophotometric analysis described.

Liquid Extract of Hydrastis. Assay Procedure. Into a glass tube 35 cm. long, 1·5 cm. in diameter with a constricted end, 20 g. of activated alumina was packed dry in portions forming an adsorption column 16 cm. long. The column was connected to a suction apparatus, 1 ml. of the liquid extract added and gentle suction was applied. Before the liquid began to disappear from above the adsorption column, 86 per cent. ethanol was added little by little to wash the alkaloids from the sides of the tube. The adsorption column was then washed with greater amounts of 86 per cent. ethanol until the percolate was alkaloid free. Usually 60 ml. of 86 per cent. ethanol were sufficient for complete washing. The yellow, clear percolate was transferred quantitatively to a 100-ml. flask and adjusted to volume with 86 per cent. ethanol.

For Berberine. An aliquot part of the percolate was diluted (e.g. 1:25) with 86 per cent. ethanol and the E 350 mμ value measured. Berberine could be calculated according to the relation $c = E/E$ (1 per cent. 1 cm.).

Where c = concentration in g. per cent.,
E (1 per cent. 1 cm.) = 694 at 350 mμ,
E = measured extinction at 350 mμ.

For Hydrastine. Another aliquot part of the ethanol percolate, usually about 25–50 ml., was distilled on the water bath until a minimum amount of the aqueous liquid remained. About 30 ml. of distilled water was added and the solution transferred quantitatively to a separating funnel.

TABLE I

COMPARISON OF RECOVERY OF BERBERINE AND HYDRASTINE FROM THEIR MIXTURES BY THE SPECTROPHOTOMETRIC AND B.P.C. 1949 METHODS

Spectrophotometric						B.P.C. 1949 gravimetric			
Prepared amounts of hydrastine-berberine mixture									
Hydrastine			Berberine			Berberine	Hydrastine		
Used g.	Recovered g.	Error per cent.	Used g.	Recovered g.	Error per cent.	Used g.	Used g.	Recovered g.	Error per cent.
0·0430	0·0420	−2·3	0·0874	0·0899	+2·8	0·478	0·2196	0·2062	−6·1
0·0340	0·0333	−2·0	0·0500	0·0500	0·0	0·402	0·1412	0·1308	−7·3
0·0580	0·0550	−5·1	0·0100	0·0100	0·0	0·685	0·1036	0·0874	−15
0·0133	0·0135	+1·5	0·0680	0·0650	−4·4	0·0995	0·198	0·190	−4·0
0·0400	0·0400	0·0	0·0160	0·0166	+3·7	0·1842	0·1784	0·1677	−5·9
0·0484	0·0465	−3·9	0·02248	0·02166	−3·6	0·1214	0·1198	0·1108	−7·5
0·0250	0·0250	0·0	0·0600	0·0600	0·0	0·1642	0·0988	0·0926	−6·2
0·0524	0·0520	−0·7	0·0174	0·0183	+5·1	0·1425	0·1014	0·0953	−6·0
0·05944	0·0600	+0·9	0·01278	0·01300	+1·7	0·1535	0·1146	0·1069	−6·7
Average error		−1·28			+0·58				−7·1

About 5 ml. of dilute ammonia and 30 ml. of ether were used for washing out the distillation flask. These washings were added to the contents of the separating funnel and hydrastine was extracted by shaking. The ether layer was separated and the aqueous layer was further extracted by shaking with three successive portions, each of 30 ml. of ether. The combined ethereal extract was washed three times each with 30 ml. of distilled water or until alkali free, dried over anhydrous sodium sulphate

TABLE II

COMPARISON OF THE B.P.C. 1949 METHOD FOR THE ESTIMATION OF HYDRASTINE AND THE SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF HYDRASTINE AND BERBERINE IN LIQUID EXTRACT OF HYDRASTIS

Spectrophotometric								B.P.C. 1949 method			
Hydrastine				Berberine				Hydrastine			
Liq. ext. g. per cent.	Added g.	Recovered g.	Error per cent.	Liq. ext. g. per cent.	Added g.	Recovered g.	Error per cent.	Liq. ext. g. per cent.	Added g.	Recovered g.	Error per cent.
2·15	0·0100	0·0100	0·00	3·06	0·0065	0·0062	−4·6	2·008	0·0676	0·0575	−14·9
2·00	0·00507	0·00500	−1·3	3·25	0·0110	0·0107	−2·7	2·010	0·0670	0·0630	−5·9
2·12	0·00507	0·00520	+2·5	3·07	0·0110	0·01109	+0·81	1·966	0·0338	0·0312	−7·6
2·00	0·00676	0·0070	+3·5	3·25	0·0138	0·0144	+4·3	1·994	0·1064	0·1000	−6·0
2·20	0·00100	0·00100	0·00	3·25	0·0194	0·0198	+2·0	1·984	0·1242	0·1152	−7·2
2·11	0·01352	0·01380	+2·0	3·20	0·0221	0·0220	−0·45	1·994	0·1643	0·1514	−7·8
Average 2·096			+1·1	3·18			−0·10	1·992			−8·2

and distilled. The residue was dissolved in 86 per cent. ethanol and transferred quantitatively to a 25 ml. flask and adjusted to volume with 86 per cent. ethanol. A portion of this ethanolic solution was diluted 1 : 10 with the same solvent, then assayed for its hydrastine content by determining its *E* 297 mμ value.

The spectrophotometric procedure was carried out on liquid extract of hydrastis as an example of hydrastis galenicals with and without the addition of known volumes of standard solutions of hydrastine and berberine in 86 per cent. ethanol. At the same time the galenical was assayed by the B.P.C. 1949 method with and without the addition of known quantities of hydrastine for comparison. The results are shown in Table II.

Conclusions

The proposed spectrophotometric method appears to be specific for hydrastine and berberine. The accuracy of the method is shown by the high recovery of both alkaloids. The procedure estimates berberine in a galenical preparation of hydrastis with great ease, a fact which makes it a delicate and rapid method for this purpose. Because of the small amount used, the time required for completing the assay is reduced to a minimum without sacrificing accuracy. The method does not necessitate the construction of a standard curve; and complete determination of both hydrastine and berberine in the galenical can be performed within three hours. The proposed assay procedure can measure very minute amounts of each of hydrastine and berberine precisely and satisfactorily.

Summary

1. Spectroscopic data for the ultra-violet absorption of hydrastine and berberine are given.

2. These enable the two alkaloids to be identified and estimated in various drug preparations.

3. The method is rapid and the resulting assay is free from complications.

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