THE SPECTROPHOTOMETRIC IDENTIFICATION AND ESTIMATION OF STRYCHNINE, BRUCINE AND MORPHINE IN VISCERA EXTRACTS

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INTRODUCTION

THE extraction of substances such as alkaloids from toxicological specimens and their identification when present in small amounts has been the subject of much study, ably summarised by Bamford,[1] Authenreith[2] and Turfitt.[3] In the case of alkaloidal extracts the detection proceeds by a series of colour reactions, each of which uses, without possibility of recovery, an appreciable aliquot of a sample often submitted in small quantity. Many of the tests yield negative results with further waste of valuable material and some are by no means specific: thus yohimbine and the alkaloids of *Gelsemium elegans* give blue colours with Fröhde's reagent so that an investigator may well be put on the wrong track in the initial stages. Furthermore impurities often interfere with colour reactions; a purification process may be required and the amount of extract reduced even more.

Turfitt (*loc. cit.*) has noted the use which may be made of spectrophotometric analysis in toxicological work. Elvidge[4] and Brustier[5] have studied the absorption spectra of many pure alkaloids and found not only that most have pronounced absorption bands in the far ultra-violet region but that these absorption bands are sufficiently specific to be used for identification.

We have applied spectrophotometry to the detection and estimation of the alkaloids, particularly strychnine, brucine and morphine, in Stas-Otto extracts from viscera and some natural products. We have standardised the procedure by measuring the absorption spectra of the pure alkaloids in ethanolic solutions and tested the method with viscera extracts containing known amounts of strychnine, brucine, and morphine. We have had in view the possibility of detecting strychnine and brucine in both purified and crude Stas-Otto extracts, the crude extracts being those obtained by the normal Stas-Otto process without subsequent purification. We have also studied the quantitative estimation of these alkaloids, both singly and when present as a mixture. Experiments have been made with Stas-Otto extracts of viscera free from alkaloids to determine the extent to which other materials may be extracted and interfere with the spectrophotometric determination.

It is not proposed to describe the methods of determining absorption spectra or the analysis of a spectral curve because these have already been dealt with adequately, e.g., by Twyman and Lothian[6] and by Brode.[7] The usual precautions were taken against solvent impurities, instrumental aberration and in the use of cells. All extinction coefficients are expressed

in the usual form of $E_{1\text{ cm.}}^{1\text{ per cent.}}$, i.e., by log I_0/I where I_0 is the intensity of the incident light and I that of the light emerging from a 1 cm. cell containing a 1 per cent. solution of the alkaloid in question, it being assumed that Beer's Law can be used to express the factor between a 1 per cent. solution and that used.

Experimental

I. General

All absorption spectra were measured with a Beckmann Spectrophotometer, Model DU, using a hydrogen discharge tube as an ultra-violet radiation source. Cells were of quartz, of accurately known cell length and the optical faces were kept perfectly clean. The spectral range examined lay between 2100Å and 3500Å. Extinction coefficients were measured at intervals of not more than 20Å, but at critical points, i.e., turning points, inflexions, etc., measurements were made at distances 5Å apart. Extinction values for many solutions were repeated several times, and the instrumental error, in the density range 0·4 to 1·0, was found to be 1 per cent. or less. In some cases solutions were prepared in duplicate and it was found that their absorption curves were practically identical, the differences not exceeding 1 per cent. except for readings taken at the lower range of the "density" drum, which were of no great significance in this work.

The chemicals used in this work were of B.P. quality and their purity was checked by a series of melting point determinations. Ethanol, used as a solvent, was allowed to stand over silver nitrate for several days with periodic shaking and was finally distilled over caustic potash. A spectrophotometric examination of the ethanol did not reveal the presence of any impurities.

The concentrations of the experimental solutions were arranged so that maximum "density" readings would not exceed 1·0. This precaution was taken to ensure accuracy, since all readings above 1·0 and below 0·4 on the "density" drum have a greater degree of error than those between 0·4 and 1·0.

II. The Absorption Spectra of Strychnine, Brucine and Morphine

Strychnine, brucine and morphine were dissolved in ethanol (95 per cent. v/v) to give solutions of 0·0025 per cent. w/v, 0·0025 per cent. w/v and 0·005 per cent. w/v respectively. The ultra-violet absorption spectra were determined and the curves are shown in Figure 1. Those for strychnine and brucine indicate pronounced absorption bands whilst the curve for morphine shows one less pronounced absorption band in the same region. The wavelengths at which maxima and minima occur for these solutions and the extinction coefficients at these wavelengths are given in Table I.

III. The Absorption Spectra of Strychnine, Brucine and Morphine Extracted from Specimens of Viscera

The Stas-Otto process which was used to extract the alkaloids from specimens of viscera has been described in many texts[1,2] and is still recog-

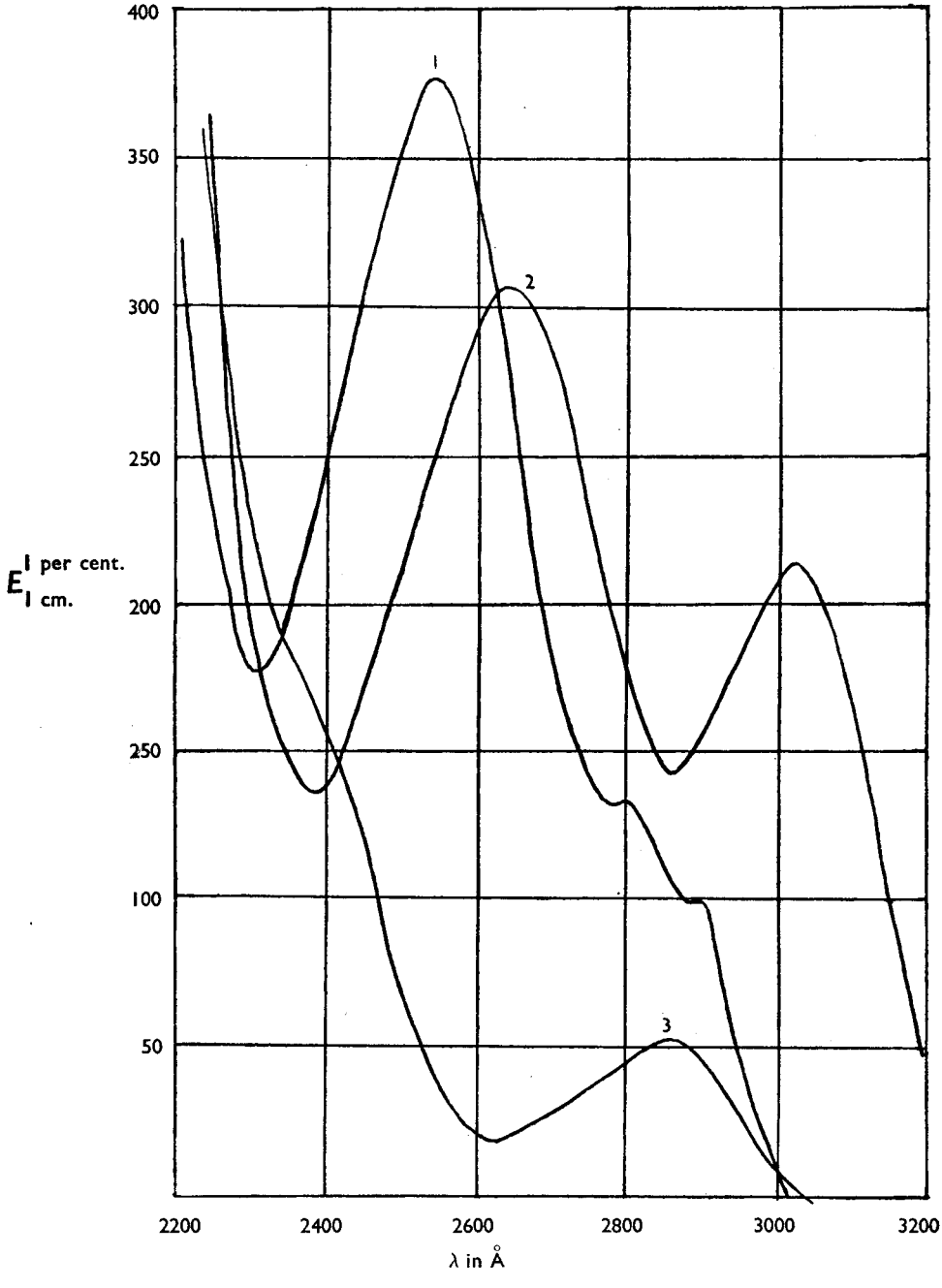

FIG. 1. Strychnine, brucine and morphine in ethanol (95 per cent.).

1. Strychnine.
2. Brucine.
3. Morphine.

TABLE I

Alkaloid	Maxima	$E^{1\%}_{1\,cm.}$	Minima	$E^{1\%}_{1\,cm.}$
Strychnine	2550Å	377	2320Å	180
	2800Å	130	2780Å	130
	2900Å	101	2880Å	100
Brucine	2640Å	307	2380Å	138
	3025Å	216	2870Å	143
Morphine	2870Å	55	2630Å	22

nised as the most satisfactory method. A brief outline of the Stas-Otto process is given below.

(*a*) The specimen of viscera is reduced to a fine state, made acid with tartaric acid and extracted with warm ethanol (90 per cent.)

(*b*) The ethanolic extract is filtered and carefully evaporated to dryness.

(*c*) The residue is extracted with absolute ethanol, cooled and filtered, and the filtrate carefully evaporated to dryness. If the residue is "dirty," the extraction with absolute ethanol is repeated.

(*d*) The final residue is taken up with water and filtered.

(*e*) The acidified aqueous solution is extracted with ether and the residual aqueous solution, made alkaline with sodium hydroxide, is ex-extracted with either chloroform or ether. This extract normally contains any strychnine and brucine present. The aqueous residue is neutralised with acid, make alkaline with ammonia and extracted with ethanolic chloroform (10 per cent. of ethanol and 90 per cent. of chloroform). This final extract contains any morphine which may be present.

The extracts obtained by alkaline chloroform and alkaline ethanolic chloroform are described in this work as "crude" extracts, i.e., extracts not subjected to further purification processes such as those described by Bamford (*loc. cit.*). The term "blank" Stas-Otto extract is used here to refer to any extract from viscera which has been examined and found to be free from alkaloidal substances.

IV. Morphine in Viscera Extracts

In the first experiments extracts were examined from routine toxicological cases which were shown to contain morphine. The extracts, both purified and crude, were dissolved in ethanol (95 per cent.) to give a solution of 0·005 per cent. w/v and the absorption curves are shown in Figure 2. It will be seen that all these curves have a pronounced $E_{max.}$ at 2870Å and $E_{min.}$ at 2630Å and the general shape of the curve gives a satisfactory qualitative test for morphine. The nature of the extract, i.e., whether purified or crude, has a profound effect on the value of the $E_{max.}$ and in some cases tends to flatten the curves at the turning points. The amount of morphine present in the extracts was determined from the values of the $E_{max.}$ and $E_{min.}$ and compared with the amount of morphine determined on the same extract by the method of the British Pharmacopœia, 1948. In general the amounts determined by the British Pharmacopœia method and by the spectroscopic method on purified morphine extracts were in good agreement but the results from "crude" extracts were open to considerable error.

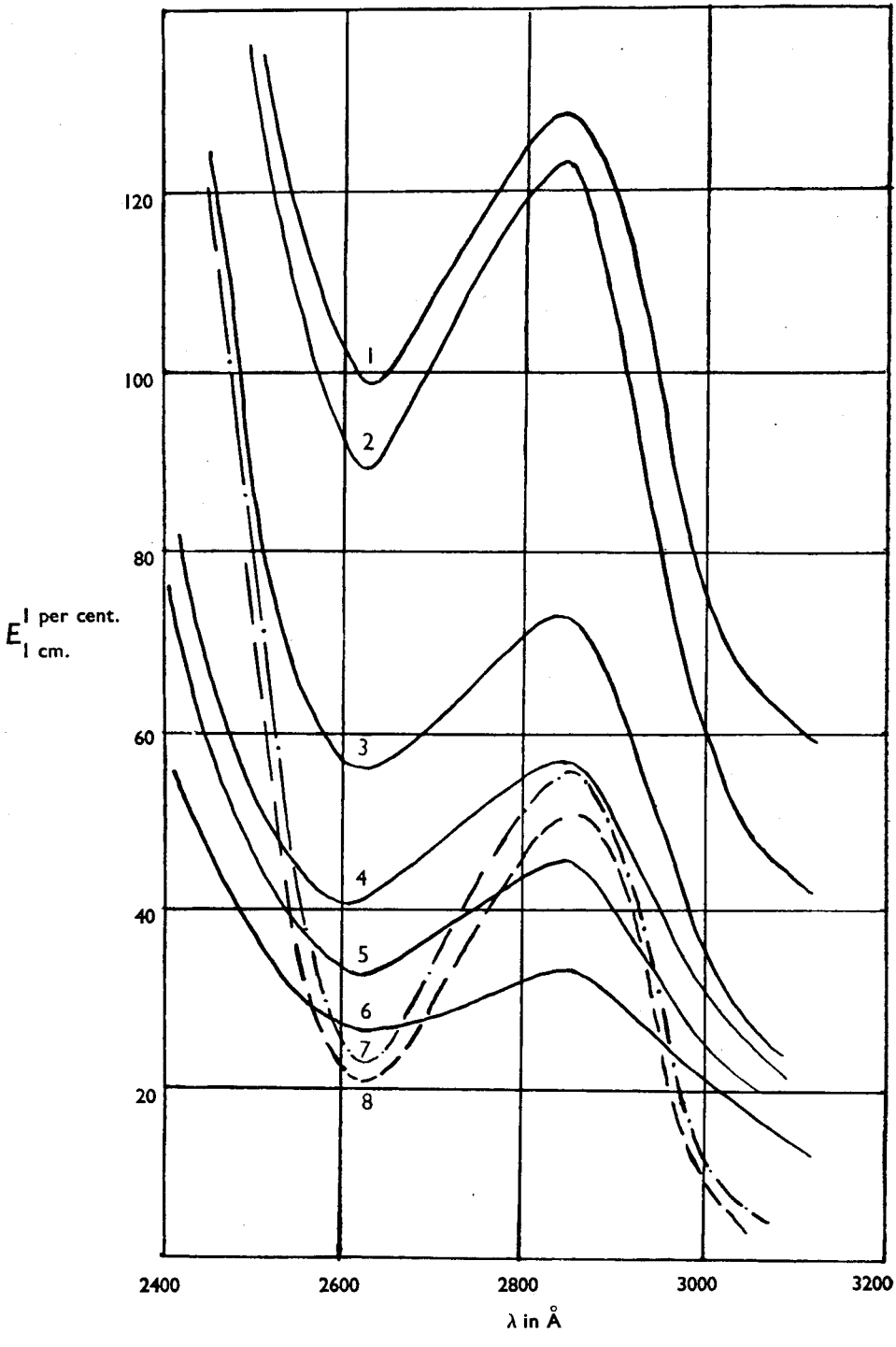

FIG. 2. Morphine in viscera extracts from toxicological cases.
1, 2, 3, 4, 5, 6 and 8. Morphine in viscera extracts both "purified" and "crude."
7. Morphine in ethanol (95 per cent.).

In the second series of experiments known amounts of morphine were added to aliquot parts of a "blank" Stas-Otto extract and the mixture dissolved in ethanol (95 per cent.) to give 0·005 per cent. w/v solutions. From the absorption spectra curves (Fig. 3) and especially from the $E_{max.}$ and $E_{min.}$ values at 2870 and 2630Å the amounts of morphine in the mixtures were calculated and are shown in Table II.

This experiment shows that the shape of the curve can be used for the qualitative detection of morphine unless the extract contains considerable amounts of extraneous matter as in curve 4: in such a case the extract

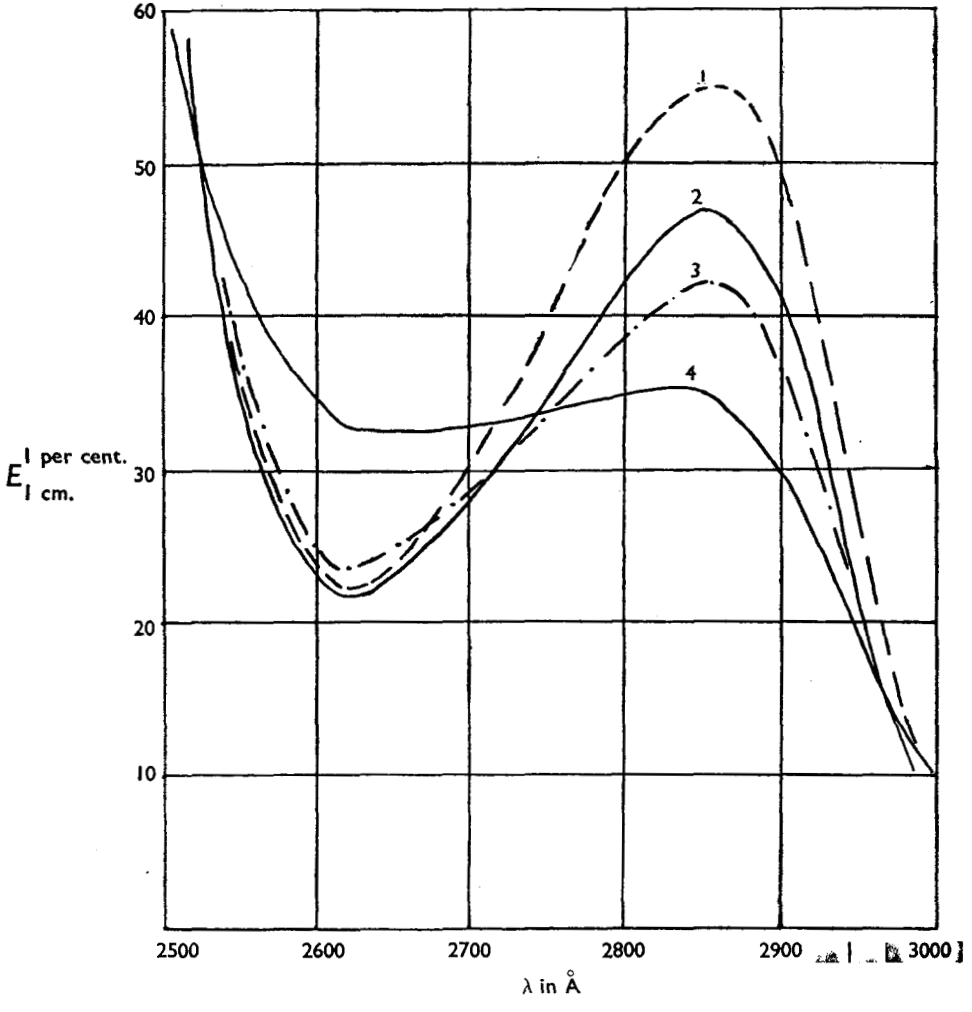

FIG. 3. Known amounts of morphine added to "blank" viscera extracts.
1. Morphine in ethanol (95 per cent.)
2. 5 mg. morphine and 1 mg. viscera extract in ethanol (95 per cent.).
3. 3 mg. morphine and 1 mg. viscera extract in ethanol (95 per cent.).
4. 1 mg. morphine and 1 mg. viscera extract in ethanol (95 per cent.).

should be cleaned up by further purification. Furthermore, the value of $E_{max.}$ at 2870Å gives a good quantitative measure of the amount of morphine present if the extract is not too impure; again it is clear that if, as with curve 4, the extract is only 50 per cent. pure, a further purification is required unless the agreement between 1 mg. of "known" morphine and 1·24 mg. "found" is sufficient for the purpose of the analysis. The $E_{min.}$ at 2630Å is of little use for quantitative work because the impurity in the extract must also contribute to the value of E at this wavelength.

TABLE II

Curve	Morphine added to 1 mg. of extract	Morphine found at 2630Å	Morphine found at 2870Å
2	5 mg.	5·7 mg.	5·0 mg.
3	3 mg.	4·2 mg.	3·0 mg.
4	1 mg.	2·9 mg.	1·24 mg.

TABLE III

Curve	Strychnine added mg.	Strychnine plus extract mg.	Strychnine found at 2320Å mg.	Strychnine found at 2550Å mg.	Strychnine found at 2800Å mg.
1	3	4·9	6·5	3·3	4·5
2	3	5·0	3·7	2·9	3·4
3	3	4·5	3·0	2·8	2·8
4	3	4·5	2·8	2·6	2·6
5	3·5	5·5	3·0	3·2	3·0

The general conclusion from these two sets of experiments is that morphine can be estimated with accuracy even when only small amounts are available, making use of the $E_{max.}$ of a purified extract.

V. Strychnine in Viscera Extracts

In the third series of experiments known amounts of strychnine were added to alkaloid-free viscera extracts and the mixture dissolved in ethanol (95 per cent.). In one case strychnine was added to liver known to be alkaloid-free and the mixture extracted by the Stas-Otto process. The absorption curves for these solutions, shown in Figure 4, exhibit the characteristic features of the curve for pure strychnine, in particular, the pronounced minimum at 2320Å and maximum at 2550Å. The subsidiary features of the strychnine curve in the region of 2800Å and of 2900Å can also be seen in the curves for the extracts. Ample qualitative evidence for the presence of strychnine is afforded and it is clear that any other substances extracted from the viscera did not affect the shape of the absorption curve to an extent sufficient to interfere with its value for qualitative detection.

The extinction values are, of course, less than those for pure strychnine because the extracts contained diluent material. A comparison of the observed values of E with those for pure strychnine at the same wave length should give the amount of strychnine in the extract, and in Table III the amount known to be in each extract is compared with that found from the values of E at 2320Å, 2550Å and 2800Å. This table shows that

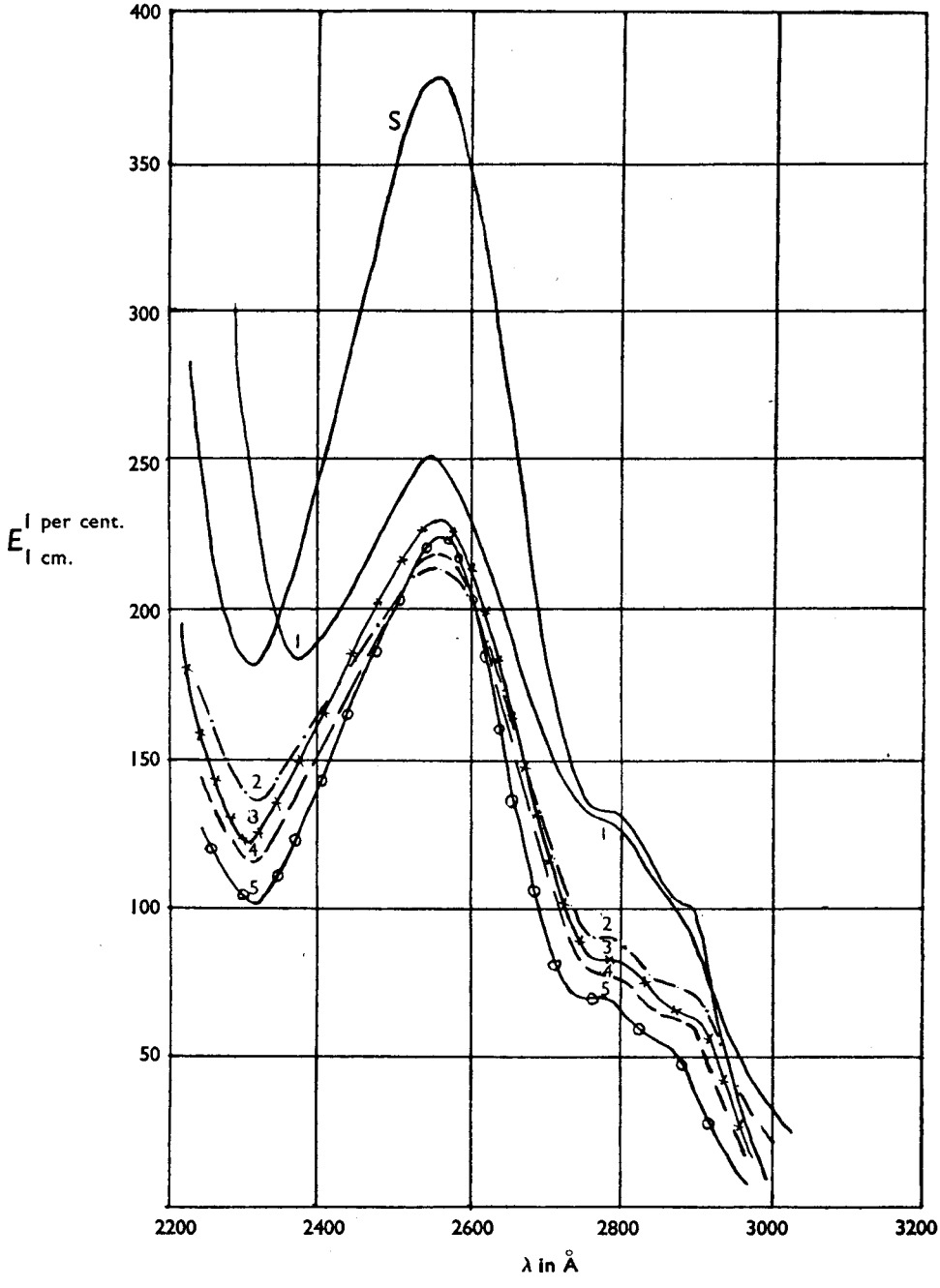

FIG. 4. Strychnine in viscera extracts.

S. Strychnine in ethanol (95 per cent.).

1–5. Strychnine in viscera extracts: dissolved in ethanol (95 per cent.).

the other material present in the extract does interfere with the quantitative estimation if the value of 2320Å is used whereas the $E_{max.}$ at 2550Å gives a good quantitative estimate of the strychnine present.

The conclusions drawn from these experiments are therefore similar to those from the experiments on morphine.

VI. The Analysis of Dilute Solutions Containing Small Amounts of Both Strychnine and Brucine

It was considered that the spectrophotometric technique would be ideally suited for the analysis of solutions containing mixtures of alkaloids in very small amounts. With this in view ethanolic solutions were prepared containing both strychnine and brucine, the ratio of strychnine to brucine ranging from 3:1 to 1:3. The solutions were diluted to contain 0·0025 per cent. of total alkaloids and were examined spectrophotometrically. The absorption curves obtained are shown in Figure 5.

These absorption curves were analysed as follows:—

(1) An approximate percentage of brucine was determined by making use of the experimentally determined extinction coefficient at 3025Å and the known extinction coefficient at 3025Å for pure brucine.

(2) The extinction coefficient for this approximate amount of brucine was calculated for a wavelength of 2550Å and subtracted from the experimental extinction coefficient at this wavelength: the residual extinction was treated as belonging to strychnine and used to calculate the amount of strychnine in the mixture.

(3) If the amount of strychnine was found to be appreciable, the brucine was recalculated, due allowance being made for the absorption due to strychnine at wavelength 3025Å.

(4) As a check, the brucine was recalculated using the $E_{max.}$ at 2640Å after allowance had been made for the absorption due to strychnine at this wavelength.

Example: Analysis of curve 3 *in Figure* 5.

Experimental extinction at 3025Å	= 156
Extinction for pure brucine at 3025Å	= 216
∴ brucine in the mixture	= 72·0 per cent.
Extinction for 72·0 per cent. of brucine at 2550Å	= 181
Experimental extinction at 2550Å	= 270
∴ Extinction due to strychnine at 2550Å	= (270–181) = 89
Extinction for pure strychnine	= 376
∴ strychnine in the mixture	= 24·0 per cent.

Check.

Experimental extinction at 2640Å	= 295
Extinction due to 24·0 per cent. of strychnine at 2640Å	= 74
∴ Extinction due to brucine at 2640Å	= (295–74) = 221
Extinction due to pure brucine at 2640Å	= 308
∴ brucine	= 72 per cent.

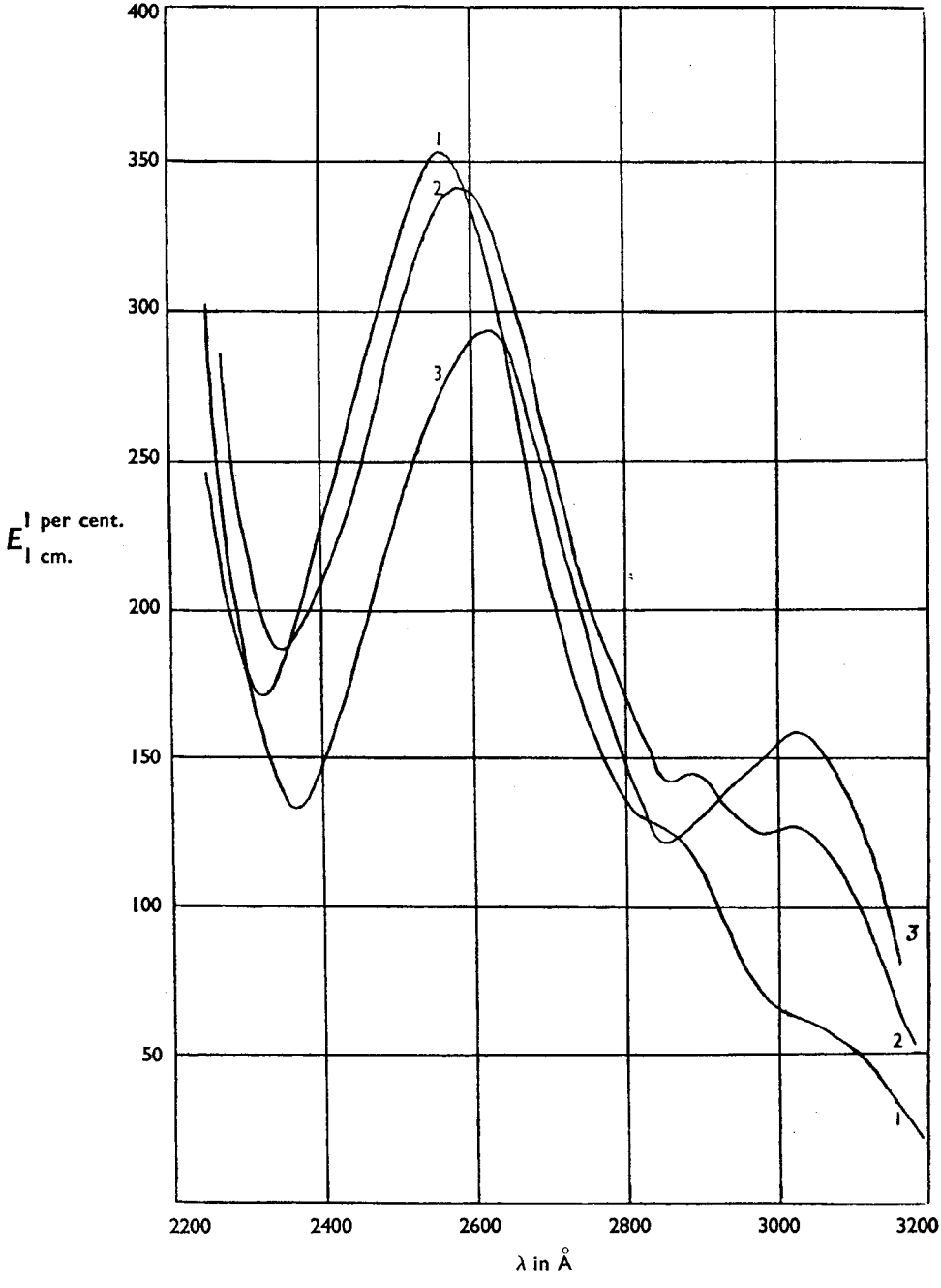

FIG. 5. Mixtures of strychnine and brucine in ethanol (95 per cent.).
1. 3·75 mg. of strychnine and 1·25 mg. of brucine in 100 ml. of ethanol.
2. 2·5 mg. of strychnine and 2·5 mg. of brucine in 100 ml. of ethanol.
3. 1·25 mg. of strychnine and 3·75 mg. of brucine in 100 ml. of ethanol.

The known and experimentally determined quantities of strychnine and brucine in the solutions for which graphs are given in Figure 5 are shown in Table IV and the values suggest that the method should be a valuable one for the analysis of this difficult mixture.

TABLE IV

ANALYTICAL RESULTS ON SOLUTIONS CONTAINING 5 MG. OF MIXED ALKALOIDS IN 100 ML. OF ETHANOL

Curve		Known amounts mg.	Found mg.
1	Strychnine	3·75	3·82
	Brucine	1·25	1·27
2	Strychnine	2·5	2·65
	Brucine	2·5	2·65
3	Strychnine	1·25	1·20
	Brucine	3·75	3·60

VII. Analysis of Stas-Otto Extracts Containing Both Strychnine and Brucine

As an extension of the above, known amounts of both strychnine and brucine were added to aliquot parts of a "blank" Stas-Otto extract. The alkaloids and extract material were dissolved in ethanol (95 per cent.), diluted to a suitable concentration and the solutions examined spectrophotometrically. The absorption curves are shown in Figure 6.

These absorption curves were analysed by the same method as that used for strychnine and brucine in pure solution and the known and experimentally determined quantities of strychnine and brucine are shown in Table V.

TABLE V

MIXTURES OF STRYCHNINE AND BRUCINE IN STAS-OTTO EXTRACTS

Curve		Known amounts mg.	Calculated mg.
1	Strychnine	3·3	3·9
	Brucine	2·0	2·0
2	Strychnine	6·6	6·2
	Brucine	4·0	4·3

DISCUSSION

These experiments do not pretend to be an exhaustive investigation; rather are they intended as an inspection of the potentialities of the spectrophotometric method in an analytical field where the nature of the sample and the amounts available have been most unfavourable to the analyst. It is hoped that by drawing attention to this method, interest may be stimulated to further experimental work in this field.

SUMMARY

1. Spectrophotometric measurements have been made on the absorption spectra of morphine, strychnine and brucine and of strychnine-brucine mixtures.

2. The work has been extended to Stas-Otto viscera extracts containing these alkaloids.

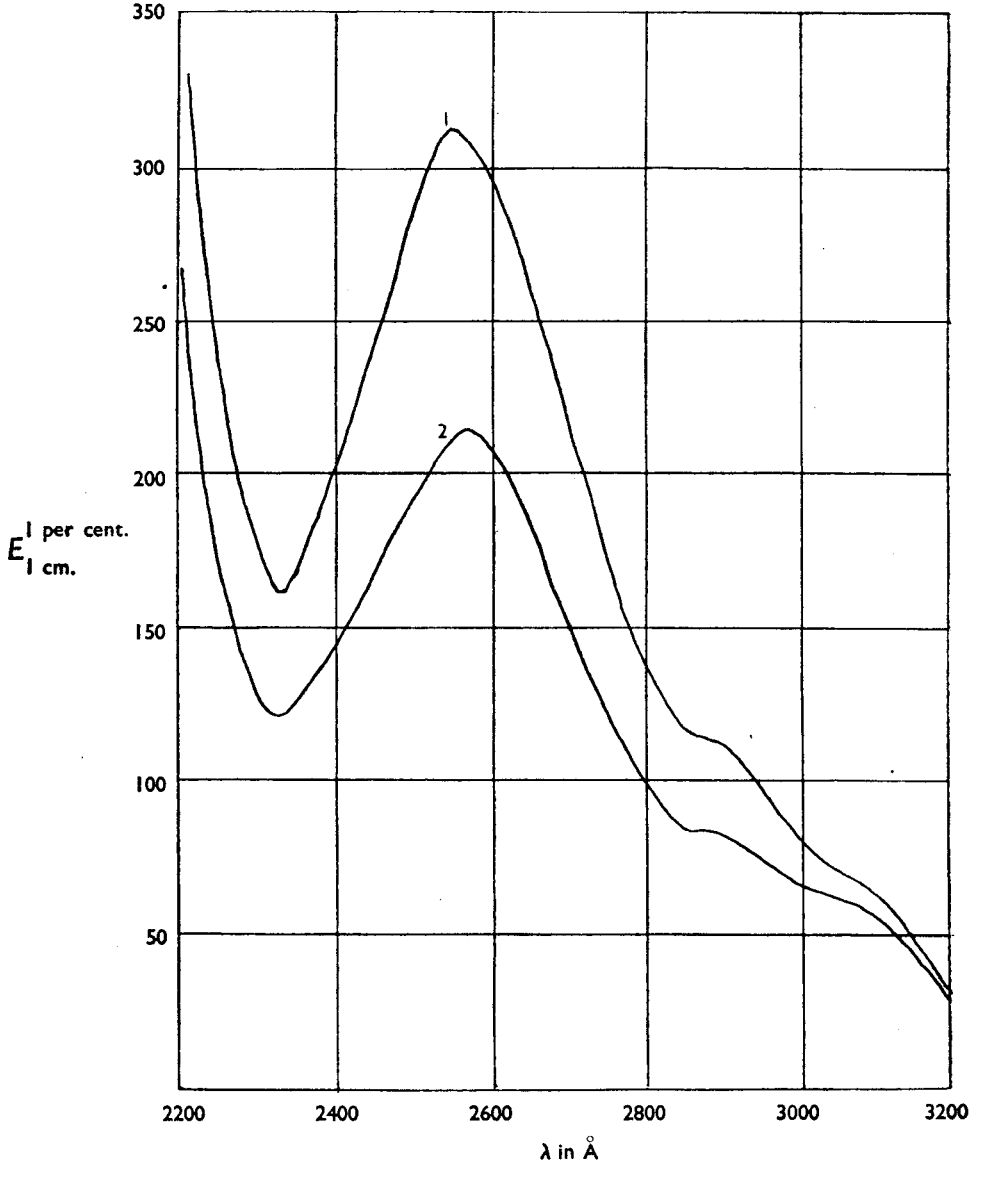

FIG. 6. Mixtures of strychnine and brucine in viscera extracts.
1. 0·0066 g. of extract containing 3·3 mg. of strychnine and 2·0 mg. of brucine.
2. 0·0168 g. of extract containing 6·6 mg. of strychnine and 4·0 mg. of brucine.

3. The use of such measurements for both qualitative and quantitative work on these substances is discussed and sufficient data have been presented to suggest that the method is one with considerable potentialities.

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