RESEARCH PAPERS

THE SPECTROPHOTOMETRIC DETECTION OF *CANNABIS SATIVA* RESIN

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Introduction

Both the dried plant fragments and the resin of *Cannabis sativa* are toxic materials which the analyst is frequently called upon to identify. They are in widespread use throughout the world under the names of Indian hemp, hashish, ganja, bhang, charras and marihuana and a description of the nature of the plant, the manner in which it is used and its physiological effects is to be found in publications by Bamford[1] and others.

The identification of *Cannabis sativa* as described by Bamford depends on a microscopic examination, if the sample is submitted in the form of plant fragments, and on a series of chemical colorimetric tests such as those devised by Beam,[2] Negm[3] and Blackie.[4] We have used these tests frequently and have found them to suffer from two major disabilities; in the first place, the microscopic proof can be used only for recognisable plant specimens whilst the colour tests are not always specific for the resin. Thus we have found that Negm's reagent can give a pale blue colour even with some normal tobacco extracts known to be free from the resin whilst in Blackie's method interfering colours are obtained from the "blank" unless very great care is taken in the preparation of pure solvents. Secondly, the methods require considerable practice on the part of even an able analyst who is encountering this material for the first time.

Jacob and Todd,[5] studying the active principles of the resin, found a substance, cannabinol, $C_{21}H_{26}O_2$, having an absorption band with a maximum at 2850Å and a molecular extinction coefficient of 16,790 and another substance, cannabidiol, $C_{21}H_{30}O_2$, with an absorption band at 2775Å and a molecular extinction coefficient of 1350. Work, Bergel and Todd[6] found that both these substances are toxic, cannabinol being much more so than cannabidiol.

We have already investigated the use of ultra-violet absorption spectra in toxicology[7] and have now examined the spectra of cannabis extracts and found that their absorption curves are characterised by a band with a pronounced maximum and minimum in the ultra-violet range of the spectrum. We have therefore applied this technique to the detection of the resin in (*a*) plant fragments, (*b*) mixtures of tobacco and cannabis and (*c*) specimens of viscera containing cannabis. Light petroleum extracts of material containing cannabis, suitably purified, showed absorption bands coincident with those obtained from the natural product and also with an absorption curve compounded from the absorption bands

due to cannabinol and cannabidiol. To standardise the technique all extracts have been examined in ethanol (95 per cent.) solution. It is not intended that the method of detection to be described should displace the already well-established procedures; rather is it hoped that spectrophotometric examination may supplement existing procedures in an analysis which is being required, unfortunately all too frequently, in many countries.

EXPERIMENTAL

I. General

All absorption spectra were measured with a Beckman spectrophoto meter, Model DU, using quartz cells and a hydrogen discharge tube as a source of ultra-violet light. The spectral range examined lay between 2100Å and 3500Å and extinction coefficients were measured at not more than 20Å intervals although at critical points, i.e., turning points etc., measurements were made at 5Å intervals. The concentrations of the solutions were adjusted so that maximum "density" readings would not exceed 1·0, because readings above 1·0 or below 0·4 on the density drum have a greater degree of error than those between 0·4 and 1·0.

The ethanol used as 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 cannabis was obtained from Police and Customs seizures, usually in the form of semi-dried leaves, stalks and flowering tops.

All extinction coefficients were expressed in the usual form of $E_{1\text{ cm.}}^{1\text{ per cent.}}$ i.e., by log I/I_0 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 cannabis extract, it being assumed that Beer's Law can be used to express the factor between a 1 per cent. solution and that used. The validity of this assumption depends on an experiment in which 5 dilutions of the same extract were measured with the following results:—

Concentration per cent.	$E_{1\text{ cm.}}^{1\text{ per cent.}}$ at 2840Å
0·005	214
0·0025	220
0·00167	231
0·001	226
0·0005	234

II. The Absorption Spectra of Cannabis sativa *Extracts*

Dried plant fragments of cannabis were powdered and extracted with light petroleum (b.pt. 60° to 80° C.). The extract was filtered, evaporated to dryness and the residue taken up in ethanol (95 per cent.) to give a solution 0·005 per cent. w/v. The absorption curve for this solution is shown as curve 3 in Figure 1. Experiments with 4 other extracts were made and the spectral data are summarised in Table I, Solutions 2 to 5.

In all these experiments normal semi-dried cannabis was used, and no attempt was made to separate leaves, stalks and flowering tops.

The data for solution 6 in Table I are taken from the absorption curve for a sample of cannabis resin which had been in the Department's poison cupboard for many years. The resin was originally of B.P. quality and, whilst the maximum absorption is found at the right wavelength, the extinction coefficient is low indicating that the sample must have undergone considerable decomposition during storage.

TABLE I

Solution	λmax.	E	λmin.	E	
1	2830Å	215	2510Å	98	saponified
2	2800Å	190	2510Å	105	not saponified
3	2800Å	188	2510Å	128	
4	2810Å	250	2510Å	135	
5	2820Å	176	2520Å	113	
6	2800Å	75	2600Å	63	

In some cases, the light petroleum extract from cannabis was saponified with ethanolic caustic soda prior to solution in ethanol. This was done in order to find out if such treatment had any influence on the shape of the absorption curve. The data for a specimen treated in this way are shown in Table I (Solution 1) whilst curve 2 in Figure I shows the absorption spectra of another saponified extract from cannabis. Saponification during the extraction process causes a slight shift in the wavelength of maximum absorption and a noticeable increase in the extinction coefficient. Moreover, there is a narrowing of the absorption band and the resulting curve gains in character so that the additional step of saponification is worth while in that it leads to a more certain identification of the absorption spectrum.

III. The Absorption Spectra of Cannabis Mixed with Tobacco

Synthetic mixtures were prepared using equal parts of dried cannabis plant fragments and tobacco. 3 types of tobacco were used: Virginian (from English cigarettes), Virginian (from American cigarettes) and Turkish tobacco.

The mixtures and the unadulterated tobaccos were extracted with light petroleum and the extract filtered. The filtrate was then extracted several times with acidified water to remove small quantities of nicotine since nicotine has a pronounced absorption band in the far ultra-violet.[8] The light petroleum extract was then evaporated to dryness, the extract taken up in ethanol (95 per cent.) and the solutions examined spectrophotometrically. The absorption curves are shown in Figure 2.

The absorption curves on the "blanks," i.e., tobacco without cannabis, were found to be devoid of pronounced absorption bands in the far ultraviolet region of the spectrum.

A partially burnt mixture of tobacco and cannabis was also extracted and treated as described above and a curve obtained (3 in Fig. 2) which

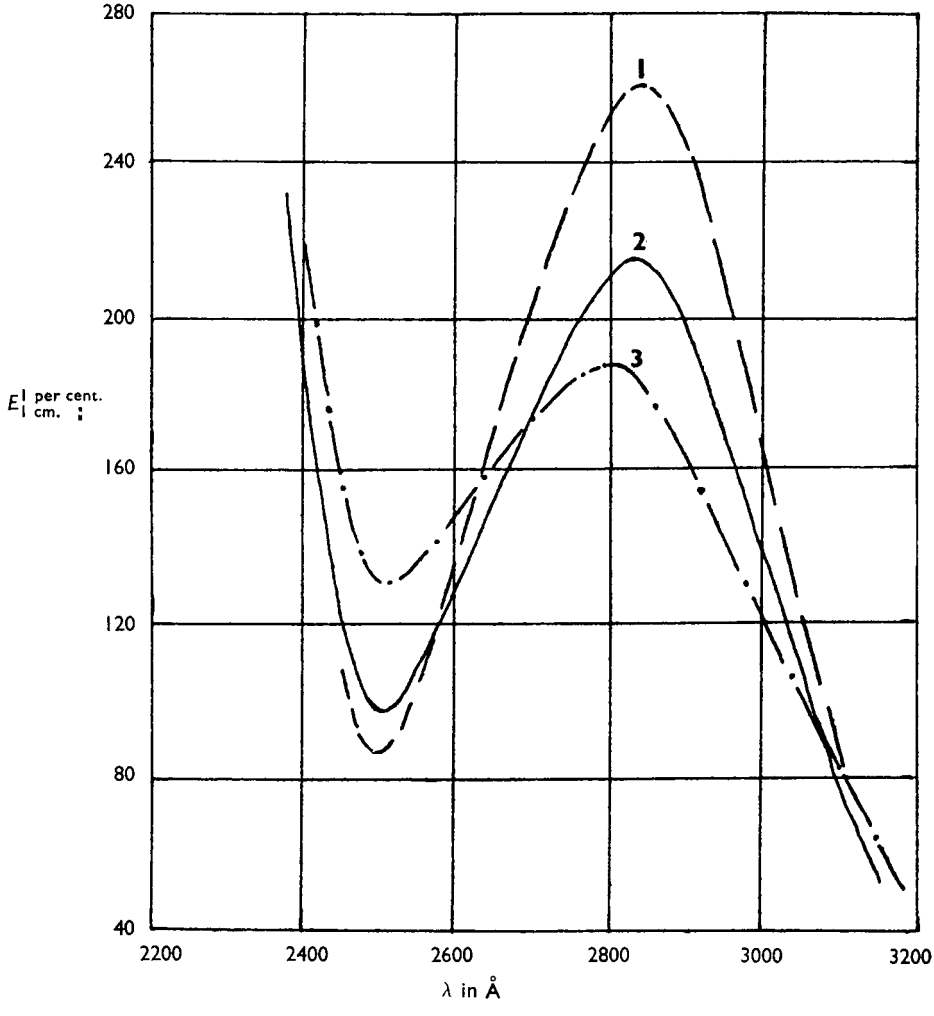

FIG. 1

1. *Cannabis sativa* extract from viscera.
2. Extract from *Cannabis sativa*—extract saponified.
3. Extract from *Cannabis sativa*—extract not saponified.

shows that the method is applicable in toxicological cases where the evidence has been partially destroyed.

IV. The Absorption Spectra of Extracts from Stomach Contents Containing Cannabis sativa

Cannabis sativa was added to several specimens of stomach and contents which had been shown to be free from toxic substances. The cannabis resin was extracted as follows: (i) The stomach and contents were extracted with light petroleum, filtered and the filtrate evaporated to dryness.

(ii) The residue was dissolved in ethanol (80 per cent.) and the solution cooled to 0° C. Most of the fatty material was precipitated and separated by rapid filtration. (iii) A small quantity of sodium hydroxide was added to the filtrate and the solution evaporated almost to dryness. (iv) The

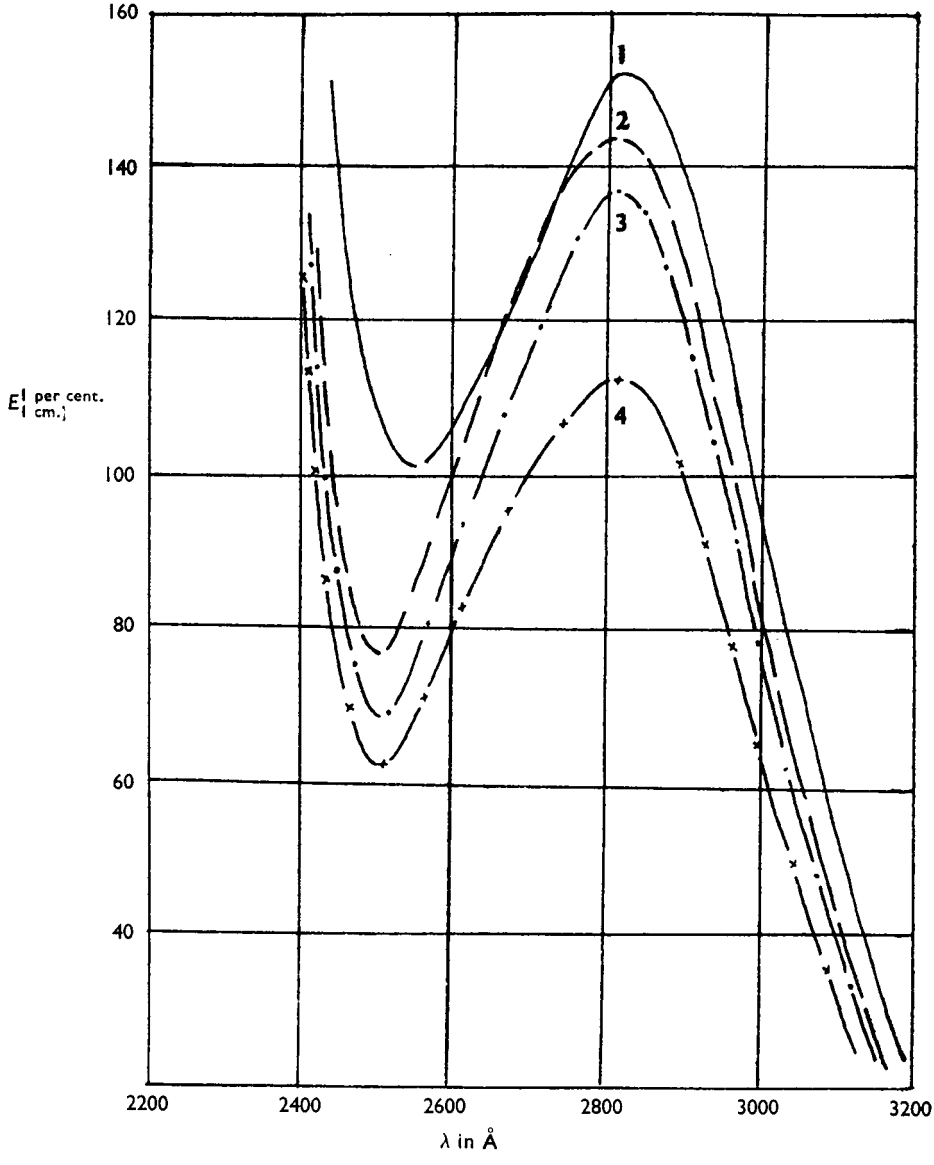

FIG. 2. Extracts of *Cannabis sativa* mixed with:

1. American tobacco.
2. English tobacco.
3. English tobacco (cigarette partially burnt).
4. Turkish tobacco.

semi-dry residue was extracted with warm light petroleum, filtered and evaporated to dryness.

This residue was taken up in ethanol (95 per cent.) and examined spectrophotometrically. The absorption curves (of which curve 1 of Figure 1 is typical) were found to be similar in shape to those for cannabis resin. The details of the absorption curves obtained from other mixtures of viscera and cannabis are shown below in Table II.

TABLE II

Sample	λmax.	E	λmin.	E
1	2840Å	260	2500	84
2	2830Å	256	2500	96

The absorption spectra were also determined for extracts which had been separated from most of the fat by solution in cold ethanol (80 per cent.) but not subjected to saponification. These extracts obviously contained some fat and therefore the solution strengths did not represent the true concentration of cannabis resin the extract. It is therefore not surprising that, whilst the absorption curves were found to have much the same shape as the curves in Figure 1, the extinction coefficients (Table III) were greatly reduced.

TABLE III

Extract	λmax.	E	λmin.	E
1. (Cooled in ethanol once)	2820Å	19	2520Å	10
2. " " "	2820Å	17	2510Å	5
3. (Cooled in ethanol twice)	2820Å	82	2510Å	27
4. " " "	2820Å	36	2530Å	30

Discussion

Todd and co-workers (*loc. cit.*) have found that cannabinol has $E_{max.}$ at 2850Å and cannabidiol at 2775Å, the molecular extinction coefficient of cannabinol being much greater than that for cannabidiol.

We have found that saponified extracts of cannabis give an absorption band with $E_{max.}$ at 2830Å $\pm$ 10Å and with extinction coefficient approximately half that of cannabinol. The absorption curve is not sharp and is probably compounded from absorption due to both cannabinol and cannabidiol. In the case of unsaponified extracts we found that the $E_{max.}$ tended to be about 2800Å and the maximum extinction coefficient was considerably less than for the saponified extract. This would indicate that the saponified extract contained considerably more cannabinol than the unsaponified extract thus tending to shift the $E_{max.}$ nearer to 2850Å and increase the extinction coefficient.

We are of the opinion that the absorption spectrum of cannabis extract is sufficiently distinctive to be used for identifying this substance and furthermore that the absorption bands are largely due to the toxic principles cannabinol and cannabidiol. The absorption curves for mixtures

of cannabis and tobacco, Figure 2, reveal the same general features as those for cannabis while the absorption curves for tobacco alone showed negligible absorption in the wave length region under study. The extraction process was designed to reduce the amount of nicotine in the extract to a minimum and the curves, Figure 2, do not reveal the presence of any nicotine. We are aware that our experiments have been restricted to a limited number of tobacco samples but in our opinion, they indicate that absorption spectra may be used to identify cannabis when mixed with tobacco and may also be used on partially burnt mixtures.

We found that direct extraction of mixtures of *Cannabis sativa* and stomach and contents with light petroleum gave an extract which contained an excessive amount of fat. Preliminary removal of fat by cooling a solution of the extract in ethanol (80 per cent.) gave an extract which still contained fatty material but the absorption spectra for these extracts showed the maximum and minimum for cannabis although the $E_{\text{max.}}$ was very low. We therefore tried a saponification technique to remove the fat residue and found that the absorption spectra for the final extracts revealed the pronounced absorption band shown by saponified *cannabis sativa* extracts. The $E_{\text{max.}}$ of the absorption curves shows that the final extracts contain very little fat or interfering absorbing substances.

We did not experiment with removing fragments of *Cannabis sativa* plant material from the stomach contents and extracting these directly. Such a step would obviate the necessity for any purification process but we feel that the more general approach, as described above, would cover all possible cases which the toxicologist might encounter. We are of the opinion that the above experimental data indicate that the spectrophotometric technique may be used to identify the presence of *Cannabis sativa* resin in stomach contents.

In this work we have not attempted to estimate quantitatively the amount of cannabinol in the various extracts obtained. However, if such was desired, the experimental curves could be analysed by using the known absorption bands of cannabinol and cannabidiol and the total amount of these substances in the extract thus determined.

This does not pretend to be an exhaustive investigation but we feel that sufficient evidence has been produced to demonstrate the potentialities of the method and to relate the absorption bands investigated with the toxic principles present in cannabis.

Summary

1. We have examined spectrophotometrically extracts obtained from several specimens of cannabis and also from mixtures with tobacco and stomach contents.

2. We have shown that these extracts possess a quite pronounced absorption band in the far ultra-violet and that this band may be used to identify such extracts. We have attributed the absorption band to cannabinol and cannabidiol which are the toxic substances present in *Cannabis sativa* resin.

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