LETTER TO THE EDITOR

Paper and Gas Chromatographic Analysis of Cannabis*

SIR,—Recent interest in the chemical and physical methods of analysis of cannabis has improved the position of the forensic chemist. Reviews of the work in this field in connection with the classical botanical-colour test procedures, paper chromatography and electrophoresis, ultra-violet and infra-red spectroscopy had been made by Farmilo (1956, 1961) and Farmilo and Genest (1959). Recently we reported the analysis of the essential oil fraction of cannabis by gas liquid partition chromatography, and the use of this procedure in the identification of cannabis (Farmilo, 1960; Martin, Smith and Farmilo, 1961). Further work has just been completed on the gas and paper chromatographic methods applied to the steam volatile oil of fresh cannabis to determine the presence of cannabidiol. The methods are sensitive and sparing of material. Gas chromatography also provides information of relative concentrations of the main cannabinols that have been identified and are now characterised. These are cannabidiol, cannabinol and tetrahydrocannabinol, which often occur in the cannabis materials. Farmilo and others (1960) have shown that the cannabidiol acid, cannabidiol, cannabinol and tetrahydrocannabinol content changes with climatic conditions from northern, through mediterranean to tropical regions. The methods now described will assist in determining the origin of cannabis.

Method of preparation of samples for paper and gas chromatography. The sample of cannabis is dried at room temperature, and, after removal of seeds and stems in the case of flowering tops, it is powdered in a mortar and transferred to a 0·1 ml. centrifuge tube. 10 mg. of hashish is sufficient: larger samples of leafy material may be required. The dry powder is wetted with a few drops of methanol from a micropipette (1 μl.). The mixture is stirred and centrifuged and the supernatant fluid is transferred to a clean dry centrifuge tube (0·1 ml.). The residue remaining in the first tube is dried in a stream of nitrogen, cooled and weighed. From the empty tube weights and the weight of the methanol used the concentrations of the sample can be obtained. The solution of cannabis in methanol is relatively free of nonacosane, and if desired can be further purified by freezing at dry-ice acetone temperatures which removes further plant waxes.

Recent work in this laboratory with the Research Specialties Co., Gas Chromatographic apparatus equipped with a beta ray ionisation detector has been carried out under the following conditions.

A methyl-silicone gum rubber (3 per cent SE-30) on Chromosorb W column packing was activated by heating at 225° for 12 hr. in the argon gas stream in the chromatographic column oven, and then at 325° for 24 hr. without the gas flow. A twenty or thirty inch column was used, at 174–190°, for the assay of the samples. The argon carrier gas flow rate was 100 ml./min. Typical sample sizes that have been used are: steam distilled oils, 0·5 μl.; light petroleum (30–60°) extracts of fresh and dry green leaf and flowering tops, 40 μg.; hashish extracts with methanol, 3 μg.; with light petroleum, 10 μg. Police seizures from northern countries involving green plant parts found in reefers, 10 to 40 μg. depending on the quality of the product. Standards of cannabidiol, cannabinol and tetrahydrocannabinol gave good chromatograms at 0·5 to 1 μg.

The method of de Ropp (1960) with minor modifications was used for paper chromatography of the cannabis extracts. The solvent was cyclohexane

* Cannabis means and includes the flowering tops (bhang, Kif, etc.), the resin (hashish, Charis, etc.) of the plant *Cannabis sativa* L. and its varieties.

saturated with dimethylformamide, and the Whatman No. 1 paper on which the sample was spotted was saturated with the lower dimethylformamide layer. The paper should not be allowed to dry before development. It is sufficient to blot the excess dimethylformamide before chromatographing. Detection and identification with diazotised *p*-nitraniline and the Gibbs reagent recommended by Korte and Seiper (1960) have been found to be satisfactory for police work. The system is affected by draughts and ambient temperature changes.

The gas chromatography retention values (RT-values) for cannabidiol, tetrahydrocannabinol and cannabinol are 9·3, 13·5 and 18·3 min. at 174°/30 in. and 109 ml./min. On standing the cannabidiol standard developed a new material which gave a peak at 8·5 min. The material was also found in an extract from Canadian hemp. Pyrahexyl has an RT-value of 23·5 min. for the main band at 180°/30 in. and 100 ml./min. The relative retention time values (RRT-values) for tetrahydrocannabinol in terms of cannabidiol are 1·83, 1·58 and 1·42 for Pyrahexyl, natural tetrahydrocannabinol and synthetic tetrahydrocannabinol respectively. When using light petroleum or methanol extracts of cannabis it is recommended that RRT-values relative to cannabinol be used for identification purposes, i.e. 0·55 and 0·76 for cannabidiol and tetrahydrocannabinol respectively.

The R_F values of cannabidiol, cannabinol and tetrahydrocannabinol are 0·12, 0·36 and 0·56. These are obtained from the orange to yellow coloured spots given by the diazo reagent. Cannabidiol acid did not stain with this reagent but gave a light blue colour under ultra-violet light at 3660 Å, the spot having an R_F value of 0·06. At least ten phenolic compounds are present in the light petroleum extract, which provides more points of comparison and identification. A complete analysis of seven samples takes 4 hr. About 20 to 40 μg. of the standards and extracts are required for identification.

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