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IDENTIFICATION OF DRUGS BY A COMBINATION OF GAS–LIQUID, PAPER AND THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The three major constituents of cannabis, namely the phenols cannabinol, cannabidiol and tetrahydrocannabinol, can be acylated by injection of a mixture of a benzene extract of cannabis and an acid anhydride into a gas chromatograph. Butyric anhydride is a suitable reagent for this purpose. Thin-layer chromatography assists in providing further parameters for identification purposes and may be used both before and after gas–liquid chromatography.

Barbiturates in a blood extract can be separated as a 'group' by a high-temperature chromatographic procedure using the cellulose of untreated filter paper as the stationary phase and water (at about 95°) as the mobile phase. The 'group' of barbiturates can then be eluted with acetone and individual barbiturates separated by gas–liquid chromatography. Further information is then obtained by forming (*within the gas chromatograph*) the trimethylsilyl derivatives of the barbiturates.

In many countries of the world, it is an offence to be in possession of the drug known as cannabis. The detection of cannabis, therefore, presents a special applied analytical problem to the forensic chemist who may be called upon to identify the drug in mixtures containing tobacco, or dust, or soil, etc. I want to show in part of this paper how this problem can be tackled by the application of GLC and TLC.

There are three major constituents of cannabis, *viz.* cannabinol, cannabidiol and tetrahydrocannabinol. Fig. 1 shows the structural formulae of these compounds from which it will be seen that they are phenolic compounds. It is, therefore, possible to subject them to the on-column acylation technique by injection into a gas chromatograph, which technique I referred to in a previous paper[1]. We have found that by injecting either butyric or hexoic anhydride together with the cannabis extract (dissolved in toluene), good resolution of the esters is obtained. Fig. 2 shows a gas chromatogram of the three constituents of cannabis and Fig. 3 illustrates the changes in retention times resulting from an injection of a mixture of cannabis extract and butyric anhydride. This particular sample contained practically no cannabidiol.

In order to identify these GLC peaks, we used two techniques. In the first technique, the compounds corresponding to each of the three peaks of the unchanged drug were collected separately following GLC. These collected fractions were then

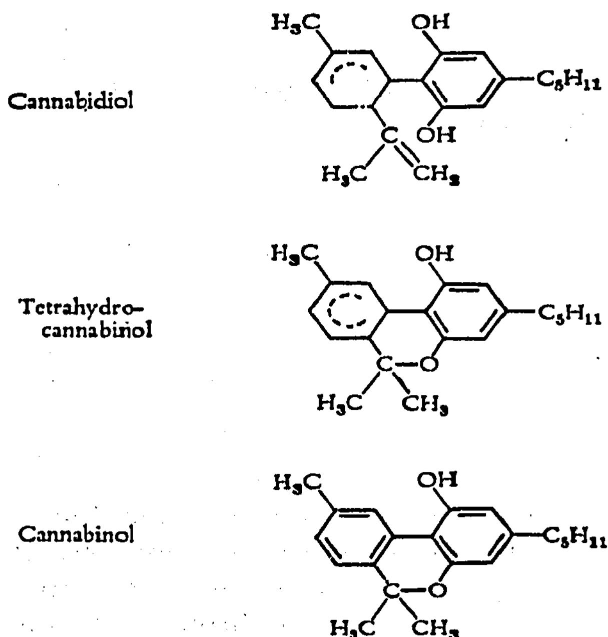

Fig. 1. Structural chemical formulae of the three major components of cannabis.

subjected to two procedures, *viz.*

(1) They were re-injected separately together with butyric anhydride.

(2) They were each subjected to TLC, using the system of KORTE AND SIEPER[2].

In the second technique, the crude cannabis extract was subjected to TLC

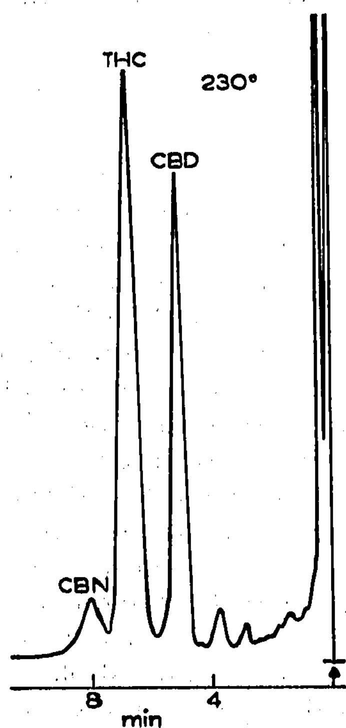

Fig. 2. Gas–liquid chromatogram of an extract of cannabis. Stainless-steel column (6 ft. × 1/8 in. I.D.) containing Chromosorb G (100–120 mesh) coated with SE-52 (see STREET[5]). Carrier gas, N_2 at 24 ml/min; column temperature, 230°; injector and detector temperatures, 280°. CBN = cannabinol; THC = tetrahydrocannabinol; CBD = cannabidiol.

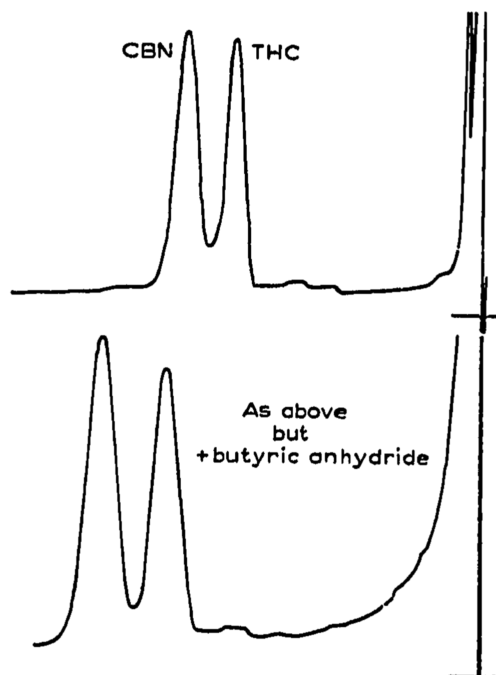

Fig. 3. Gas–liquid chromatograms of an extract of cannabis showing the 'peak shifts' obtained by injecting butyric anhydride together with the extract *in the same syringe*. Conditions and abbreviations similar to those in Fig. 2.

(again using the system of KORTE AND SIEPER). Fig. 4 shows that three spots are visible after spraying with Fast Blue salt B (di-*o*-anisidine tetrazolium chloride). In some extracts, a fourth spot is visible between the origin and the cannabidiol—this is probably due to cannabidiolic acid. An area of silica gel corresponding to the R_F

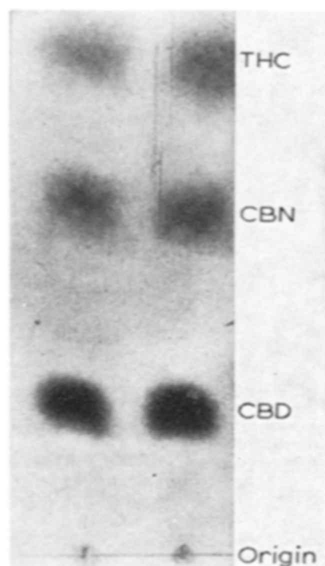

Fig. 4. Thin-layer chromatogram of an extract of cannabis showing separation of the three major constituents using the TLC system of KORTE AND SIEPER[2]. Abbreviations, see legend to Fig. 2.

value of each of these spots was scraped from the TLC plate and extracted with toluene. This toluene extract was then injected into a gas chromatograph (a) alone, and (b) together with butyric anhydride. In this way, the correspondence of the GLC peaks and the TLC spots was established.

As a further example of the combination of two different forms of chromatography, I had intended to refer to the use of GLC with our elevated temperature reversed-phase paper system[3,4]. But, when paper chromatography is used before GLC, tributyrin interferes with the GLC run. However, an interesting procedure arises when tributyrin is omitted, and the system used merely to separate 'unwanted' material from any drugs present.

In this way, barbiturates are readily detected and characterised in a blood sample. Direct extraction of slightly acidified blood with ether is used. It will be appreciated that to produce unequivocal results a direct ether extract of blood is not clean enough for immediate injection into a gas chromatograph. The ether extract is evaporated to dryness and spotted on to a piece of filter paper, which is then placed in a beaker containing a little water at about 95°. The beaker is kept in an oven. After only 10 min, the water has risen some 5 in. up the paper and the barbiturates (R_F about 0.8) are moved well away from interfering materials which remain at or near the origin. There is, of course, no resolution of the different barbiturates as is the case when the paper is impregnated with tributyrin.

The area of paper containing the barbiturates is cut out and refluxed for about 5 min with acetone. The acetone extract is evaporated and subjected to GLC. Further help in identification is obtained by injecting the acetone extract together with N,O-bis-trimethylsilylacetamide (BSA), thus forming the trimethylsilyl derivatives of the barbiturates[1]. The possibility of extending the procedure to other types of drugs is being investigated.

I hope that the two examples I have given will serve to illustrate the usefulness of combining GLC with TLC and with PC in the identification of drugs.

REFERENCES

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DISCUSSION

IRVINE: I would like to ask Dr. STREET whether he has applied automated transfer of all components from one chromatographic system to a second system, in the analysis of Cannabis substances. Would you care to comment on the possible advantages and disadvantages of automated transfer in this area of chromatography?

STREET: My answer to this is simple. No! I have not applied automated procedures. I think if the raw material contains a large number of components, then automation may be an advantage, but otherwise I think it is not necessary.