Gas chromatography of Indian hemp (*Cannabis sativa* L.)

SIR,—The use of gas chromatographic analysis for characterizing preparations of *Cannabis sativa* L. has recently been described by Betts & Holloway (1967) and Heaysman, Walker & Lewis (1967). We have also applied this technique to the detection of cannabis preparations as a complement to the thin-layer chromatographic method of Caddy & Fish (1967).

In agreement with Heaysman & others (1967) we found a combination of inert supports and low percentages of polar stationary phases, coupled with moderate oven temperatures to give the most satisfactory resolution. We found, too, that silicone polymers offer worthwhile advantages of stability and column life over the non-silicone polymeric materials.

We used columns of XE-60, a cyanoethyl silicone polymer; either 4% on Chromosorb W-AW-DMCS-100/120 mesh or 1% on Chromosorb G, AW-DMCS-100/120 mesh. These we found superior to those incorporating SE-30 (Betts & Holloway, 1967; Claussen, Borger & Korte, 1966), SE-52 (Claussen & others, 1966) and OV-17 (Lerner & Zeffert, 1966). Under the stated conditions, symmetrical and reproducible peaks are obtained for unmodified phenolic constituents extracted by chloroform maceration or percolation from 50–100 mg amounts of cannabis preparations (Fig. 1A). Peaks were identified by the use of reference compounds kindly supplied by Dr. U. Claussen, Organic Chemistry Institute, Bonn and also with compounds we have separated on thin-layer chromatograms.

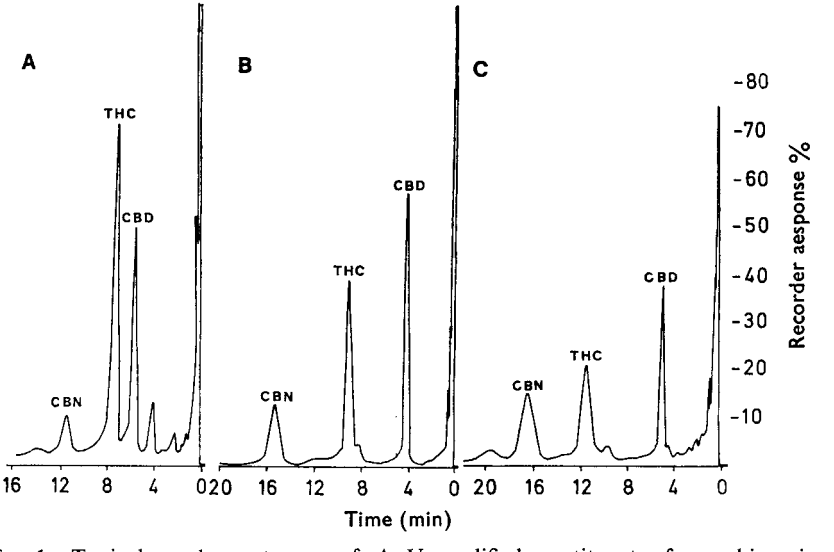

FIG. 1. Typical gas chromatogram of: A. Unmodified constituents of cannabis resin residue from 50 mg in 50 μl chloroform − 1 μl injected). Conditions: Perkin-Elmer F-11 Mk 1 with FID. Spiral glass column 6 ft × 0·3 mm i.d., 4% XE-60 on Chromosorb W. Oven 200°, injection block 250°. Nitrogen (oxygen free) 65 ml/min at Pi 45 psig, hydrogen 18 psig, air 40 psig. Attentuation 20 × 10^2. Chart speed 15 in/hr. B. Trimethylsilyl derivatives of cannabis constituents. Conditions differing from A: 1% XE-60 on Chromosorb G. Oven 165°, injection block 200°. C. Trifluoroacetyl derivatives of cannabis constituents. Conditions differing from A: Oven 150°, injection block 200°. CBD = cannabidiol, THC = tetrahydrocannabinol, CBN = cannabinol.

The resolution is improved by use of either trimethylsilyl ethers (Fig. 1B) or trifluoroacetyl esters (Fig. 1C). The silylation was essentially as described by Makita & Wells (1963), except that we used equal quantities of the two silylating reagents added to the drug residue dissolved in an equal volume of anhydrous pyridine or anhydrous isopropylamine (50–100 μl). Reaction was practically instantaneous, small aliquots being injected after centrifuging to sediment ammonium chloride. There was no difference in the reaction rates of the differing cannabinols.

The repeated use of TMSi-ethers and injection of excess silylating reagents results in contamination of a flame-ionization detector with silicates. Deposition can be delayed appreciably by injection of the isolated ethers in anhydrous hexane or carbon disulphide, but molecules containing silica are still being injected. This led us to the use of trifluoroacetyl esters. These derivatives have been used as adjuncts to the gas chromatography of steroids (van den Heuvel, Sjövall & Horning, 1961) and polar nitrogen containing compounds such as aromatic amines (Dove, 1967). They offer a suitable alternative to the TMSi-ethers and would have the further advantage of being detectable in minute amounts using an electron capture detector. Preparation is effected by refluxing the residue with 0·1 ml trifluoroacetic anhydride and 0·5–1 mg anhydrous sodium acetate, at 70° on a water-bath for 10 min. The excess fluids are driven off with a steady stream of dry air and the residue dissolved in dry acetone for gas chromatography.

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References

Betts, T. J. & Holloway, P. J. (1967). *J. Pharm. Pharmac.*, **19**, *Suppl.* 97S–102S.
Caddy, B. & Fish, F. (1967). *J. Chromat.*, **31** (2), 587.
Claussen, U., Borger, W. & Korte, F. (1966). *Justus Liebigs Annln Chem.*, **693** (1), 158–164.
Dove, R. A. (1967). *Analyt. Chem.*, **39**, 1188–1190.
Heaysman, L. T., Walker, E. A. & Lewis, D. T. (1967). *Analyst, Lond.*, **29**, 450–455.
Heuvel, van den W. J. A., Sjövall, J. & Horning, E. C. (1961). *Biochim. biophys. Acta*, **48**, 596–599.
Lerner, M. & Zeffert, J. T. (1966). *Chem. Engng News*, **44** (53), 14.
Makita, M. & Wells, W. (1963). *Analyt. Biochem.*, **5**, 523.