perature for 20 hr. The mixture was filtered, and the solids were washed with methanol. The combined filtrate and washings were concentrated *in vacuo*. Analytical TLC of the extract (two developments) showed psilocybin (R_f 0.27) and the slower compound (R_f 0.22). Both spots exhibited the same dark-blue coloration when sprayed with 2% 4-dimethylaminobenzaldehyde in ethanol and exposed to hydrogen chloride vapors. Trace amounts of psilocin were also detected.

The concentrated residue was preparatively chromatographed on a 1.0-mm layer of silica gel in the described solvent system. A band containing the two components was removed from the plate, and the compounds were eluted with methanol containing 5% aqueous ammonia. The eluate was concentrated under reduced pressure, and the residue was rechromatographed on a 0.25-mm layer of silica gel to resolve the two components. Two developments in the propanol–ammonia system followed by elution of the bands afforded psilocybin (3 mg, 0.36%), mp 175–180° [lit. (15) mp 185–195°] and its slower-moving counterpart (1 mg, 0.12%), mp 245–248° [lit. (14) mp 254–258°]. Carpophores of *P. pelliculosa* were extracted in a similar manner to give 0.08% psilocybin. Traces of psilocin were also detected.

RESULTS AND DISCUSSION

The UV spectra of the two compounds were superimposable, with λ_{max} (methanol) 290 (ϵ 4000), 280 (sh)(5000), 268 (6300), and 222 (40,000) nm, typical of psilocybin (4). The mass spectra were consistent with published data for psilocybin (11) and baeocystin (13). Psilocybin showed peaks at *m/e* 204 (relative abundance, 19%), 160 (4), 159 (3), 146 (6), 130 (3), 117 (2), and 58 (100). Baeocystin showed peaks at 190 (8), 160 (4), 159 (4), 147 (16), 146 (14), 130 (4), 117 (5), and 44 (100).

Each compound exhibited typical fragmentations of 3-indolylethylamines (16), *e.g.*, a base peak arising from β-bond fission of the ethylamine side chain (psilocybin, *m/e* 58; and baeocystin, *m/e* 44). While neither compound showed a parent peak, both showed signals for the dephosphorylated species (psilocybin, *m/e* 204; and baeocystin, *m/e* 190). The concentration of psilocybin in *P. semilanceata* was consistent with isolated yields reported from European collections (9, 11).

In agreement with Leung and Paul (14), baeocystin was not detected in *P. pelliculosa*. Psilocybin (but not psilocin) has been reported previously in this species (17).

Preliminary investigations suggest the presence of baeocystin in other species and genera. In fact, Leung and Paul (13) and Benedict (18) suggested that baeocystin may have been the unidentified compound observed by Stein *et al.* (19) in a species of *Panaeolus*.

Pharmacological effects of the analogs of psilocybin have not been studied.

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ˣ To whom inquiries should be directed.

Simple Device for GLC Separations of Cannabinoids Using a Surface-Coated Open Tube Column without Stream Splitting

N. K. McCALLUM ˣ and E. R. CAIRNS

Abstract □ A simple device that allows the GLC analysis of greater than 10 μl of sample solution on capillary columns is described. The conditions necessary for application of this device to the quantitative analysis of cannabinoids are elaborated.

Keyphrases □ Cannabinoids—GLC analysis, device using a surface-coated open tube column □ GLC—analysis, cannabinoids, device using a surface-coated open tube column □ Marijuana—GLC analysis of constituents, device using a surface-coated open tube column

GLC is the method of choice for rapid qualitative and quantitative analysis of marijuana constituents. Much literature (1) is devoted to the use of this analytical technique with packed columns. Several investigators, however, reported problems with packed GLC columns for these analyses. Some long chain alkanes have similar re-

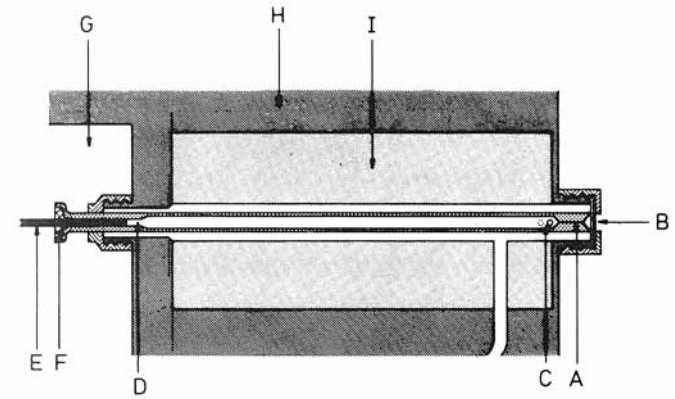

Figure 1—*Inlet port design. (See text.)*

tention times to cannabinoids and can interfere with their analyses (2). Similarly, the retention times of cannabichromene and cannabidiol are the same on most columns, and many reported analytical values for cannabidiol may be suspect for this reason (3).

Such analytical problems generally necessitate an extra analytical step, such as a preliminary chromatographic cleanup or derivatization. However, high-resolution GLC work (4) indicates that interferences by minor constituents are probably more the rule than the exception. Therefore, use of high-resolution capillary columns would be a simpler way of avoiding this problem.

DISCUSSION

Capillary columns have been used for the separation of the naturally occurring volatile terpenes (5) and cannabinoids (4, 6, 7) of *Cannabis*. Unfortunately, the use of capillary columns imposes severe restrictions on the volume of solutions that can be analyzed (0.1–1.0 μl, depending on the column diameter), so a stream splitter is generally used in conjunction with the injection system. Although stream splitters are adequate for qualitative analyses, they can introduce errors into quantitative work (8).

An alternative to the direct injection of very small quantities of solution for quantitative analysis was reported (9). This method allows the direct injection of larger quantities (usually ~0.3 μl) of the solution into an inlet port, designed so that the large amount of solvent vapor initially prevents migration of the sample components, concentrates them to 1% of the original length of the solvent plug, and allows them to be eluted after most solvent has disappeared. The theory of operation was discussed in detail (10).

The design of the inlet port is such that contact of solvent vapors with the septum is reduced to a minimum (solvent bleed from the septum being an important, undesirable factor), and all dead volumes are eliminated (9). A careful choice of solvents is also necessary to utilize the effect; the wrong solvent gives rise to broadened and misshapen peaks (10). Prolonged use of this system has not been found to affect the column life adversely (10).

The advantages of this system are clear: (*a*) utilization of the solvent to produce good resolution rather than complicated procedures to eliminate the solvent, (*b*) greatly improved sensitivity as a natural corollary to improved resolution and the injection of larger samples, and (*c*) strictly quantitative analyses without injection of very small volumes of solution. The disadvantages are designing and building the relatively complicated inlet port system and then finding the optimum solvent and conditions.

This paper presents an alternative, considerably simplified, all-glass system, which can be fabricated by most chemists of average glass-blowing ability. The described system was successfully used for the analysis of hydrocarbons and, in particular, cannabinoids.

EXPERIMENTAL

Inlet Port (Fig. 1)—The capillary (A) was chosen so that its inside diameter was a "snug" fit for the injection syringe needle and its end

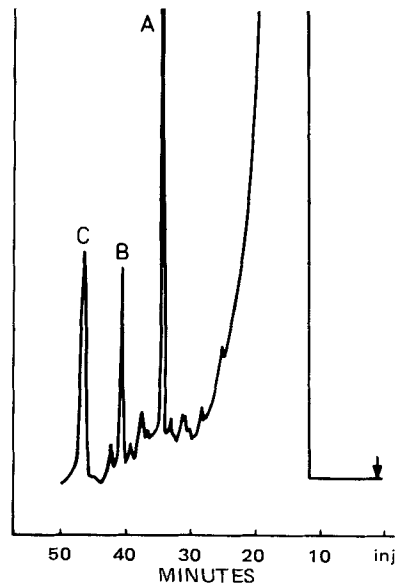

Figure 2—*Gas chromatogram of* Cannabis *sample in methyl laurate. Key: A, cannabidiol; B, Δ^9-tetrahydrocannabinol; and C, cannabinol.*

rested against the septum (B) to eliminate back-diffusion of solvent vapor. The carrier gas entered the system by two holes (C) at the base of the capillary.

The internal diameter of the exit (D) was 1.5 mm, providing an almost exact fit for the glass-lined steel tubing (E) leading to the column. The final seal at the exit was a cut-down, slightly compressed septum (F). Areas G, H, and I are the oven, oven wall, and inlet heater, respectively.

Capillary Column—The glass surface-coated open tube column[1] (70 m × 0.5 mm i.d.) was coated with SE-30, silanized, and deactivated. A "T" piece[1] for makeup gas was fitted at the exit of the capillary column, and connection with the detector port was by means of glass-lined steel tubing[1] to glass tubing (1.5 mm i.d.), using a septum for the final seal as was described for the inlet port.

Operation—A carrier gas flow of about 4 ml/min of helium was used. The makeup gas was nitrogen (~25 ml/min). A light petroleum *Cannabis* extract[2] was evaporated and taken up in the appropriate solvent to a concentration of about 0.1 mg/ml. The sample was then withdrawn completely into the barrel of the syringe, and the GLC[3] septum was penetrated to the full extent of the syringe needle (5–7 cm) and then released. After 2–3 sec, the plunger was withdrawn so that any solvent residues were taken back into the barrel of the syringe; then the syringe was withdrawn from the septum.

Sample sizes of 3 μl were taken when different solvents were being compared; 1–10-μl methyl laurate solutions were selected as the best solvents for these separations. The three major cannabinoids in the extracts (Fig. 2) were identified by comparison with authentic samples. Confirmation of these identities was provided by comparison of the derivatized and underivatized samples with authentic samples on conventional OV-1 and OV-225 columns.

RESULTS

The dimensions of the inlet system appear to have surprisingly little effect on efficiency. Vaporization chambers that were 15 cm × 1.5–3.5 mm i.d. were used and compared to 6-cm × 2.5–4-mm i.d. chambers used previously (9). The system is suitable for large sample sizes (up to 10 μl without sacrificing resolution), and differing dimensions of the oven wall and inlet heater for different models of gas chromatographs apparently present no problem for inlet design.

The suppliers of the capillary column determined its effective theoretical plates (N_{eff}) to be 43,000 for the C_{24} *n*-alkane in methylene chlo-

[1] Supplied by the Scientific Glass Engineering Pty. Ltd., N. Melbourne, Victoria, Australia 3051.

[2] A sample, identified and supplied by Mr. Peter D. Wilson of the Forensic Section, Chemistry Division, Department of Scientific and Industrial Research, Private Bag, Petone, New Zealand, is being held under Code No. AW 4520 by the Forensic Section.

[3] Tracor model 550 with flame-ionization detection.

ride (0.1 μl). When this inlet system was used with the C_{18} *n*-alkane in C_{12} *n*-alkane (5 μl), N_{eff} = 40,000 was obtained. However, GLC of cannabinoids in the higher alkanes produced very poor resolution with considerable peak tailing. Alternative solvents were tested (*e.g.*, *n*-alkanes up to C_{20}, branched chain alkanes up to C_{10}, higher boiling alkenes, alcohols, and ketones), and only fatty acid esters provided acceptable resolution. The best was methyl laurate, which yielded N_{eff} = 25,000 (Fig. 2).

The temperature of the inlet port is critical for good resolution. If the temperature is too low—by as little as 10–15°—no recognizable chromatogram will be achieved. Operating conditions for the cannabinoids in methyl laurate were: oven temperature, 230°; and inlet temperature, 250°.

The N_{eff} for cannabinoids is only 63% that achieved for alkanes. This reduction in efficiency apparently is due, at least in part, to support effects (11); the possibility exists that even more efficient separations of cannabinoids can be achieved with a different stationary phase in combination with another solvent. Different stationary phases may, of course, also provide more convenient elution orders to suit particular problems.

The main point to be noted is that the described device now permits the application of capillary columns (with its attendant 10-fold plus improvement in resolution over conventional columns) to cannabinoid analyses; this system should be of considerable use for biological as well as plant extracts. Other stationary phases and solvents possibly will be applicable to GLC of derivatized cannabinoids. This variety would allow further improvements in the sensitivity of detection of cannabinoids when utilizing electron-capture (12) and flame-ionization (13) detectors.

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[x] To whom inquiries should be directed.

Dihydralazine Sulfate Analysis Using 2-Methyl-3-nitropyridine-6-carboxaldehyde

A. F. YOUSSEF [x], S. A. IBRAHIM, and S. R. ELSHABOURI

Abstract □ A sensitive, selective colorimetric assay was developed for the quantitative analysis of dihydralazine sulfate. The method is based on the interaction of buffered (pH 4) dihydralazine sulfate with a methanolic solution of 2-methyl-3-nitropyridine-6-carboxaldehyde upon heating to give an orange color. This color can be quantified spectrophotometrically at 450 nm, with a lower limit of detection of 1 μg/ml. The color is stable for at least 24 hr. There is no interference from other drugs likely to be present along with dihydralazine sulfate and common excipients. The method was used successfully for the determination of dihydralazine sulfate in combination with other drugs in different commercial tablets. The developed method was applicable as a stability-indicating assay.

Keyphrases □ Dihydralazine sulfate—colorimetric analysis using 2-methyl-3-nitropyridine-6-carboxaldehyde, prepared samples □ Colorimetry—analysis, dihydralazine sulfate in prepared samples □ 2-Methyl-3-nitropyridine-6-carboxaldehyde—color reagent in analysis of dihydralazine sulfate in prepared samples □ Antihypertensive agents—dihydralazine sulfate, colorimetric analysis in prepared samples

Various methods for the analysis of dihydralazine sulfate (1,4-dihydrazinophthalazine) have been reported (1–11). The bromometric assay for pure dihydralazine sulfate used potassium dichromate (1) or potassium permanganate (2) as the titrant in the presence of potassium bromide. The dihydralazinebenzoic acid–bivalent metal complexes were estimated by titration with edetic acid (3, 4); the dihydralazine–copper thiocyanate complex was estimated by titration with silver nitrate (5).

The reported argentimetric procedures depend on either the estimation of excess silver nitrate added to the buffered solution of dihydralazine at pH 5–6 (6) or stoichiometric determination of metallic silver separated after the addition of ammoniacal silver nitrate (7). Direct potentiometric titration of pure dihydralazine sulfate and other hydrazine derivatives, using chloramine-T in an acidic medium, was reported (8). A coulometric method for dihydralazine, using electrolytically generated bromine, also was reported (9).

Colorimetric methods were developed for the estimation of dihydralazine sulfate in the pure form and in tablets, depending on the formation of a chromogenic product with ammonium molybdate (6) and ferric ion (10); the lower limits of detection were 70 and 150 μg, respectively. Also, a colorimetric method was reported