CHROM. 5974

Minor components of cannabis resin

III. Comparative gas chromatographic analysis of hashish

In the early 1960's gas chromatography was introduced into the analysis of cannabis resin[1]. A method for rapid and relatively accurate quantitative determination of the main components, cannabidiol (CBD), tetrahydrocannabinol (THC) and cannabinol (CBN), thus became available. The question soon arose whether the amounts of these components were in some way correlated to the geographical origin of the cannabis material analyzed.

Several papers have been published indicating that such a correlation may exist[2,3]. However, the problem is very complicated because many factors influence the chemical composition of the cannabis resin, *e.g.* the genetic properties of the plant, the soil, the climate, the state of maturity at the time of harvest, as well as the storage time and the storage conditions. Investigations have been carried out, indicating that the genetic properties are a dominating factor[4,5]. As it can be safely assumed that seeds are exchanged between different parts of the world for illegal cultivation,

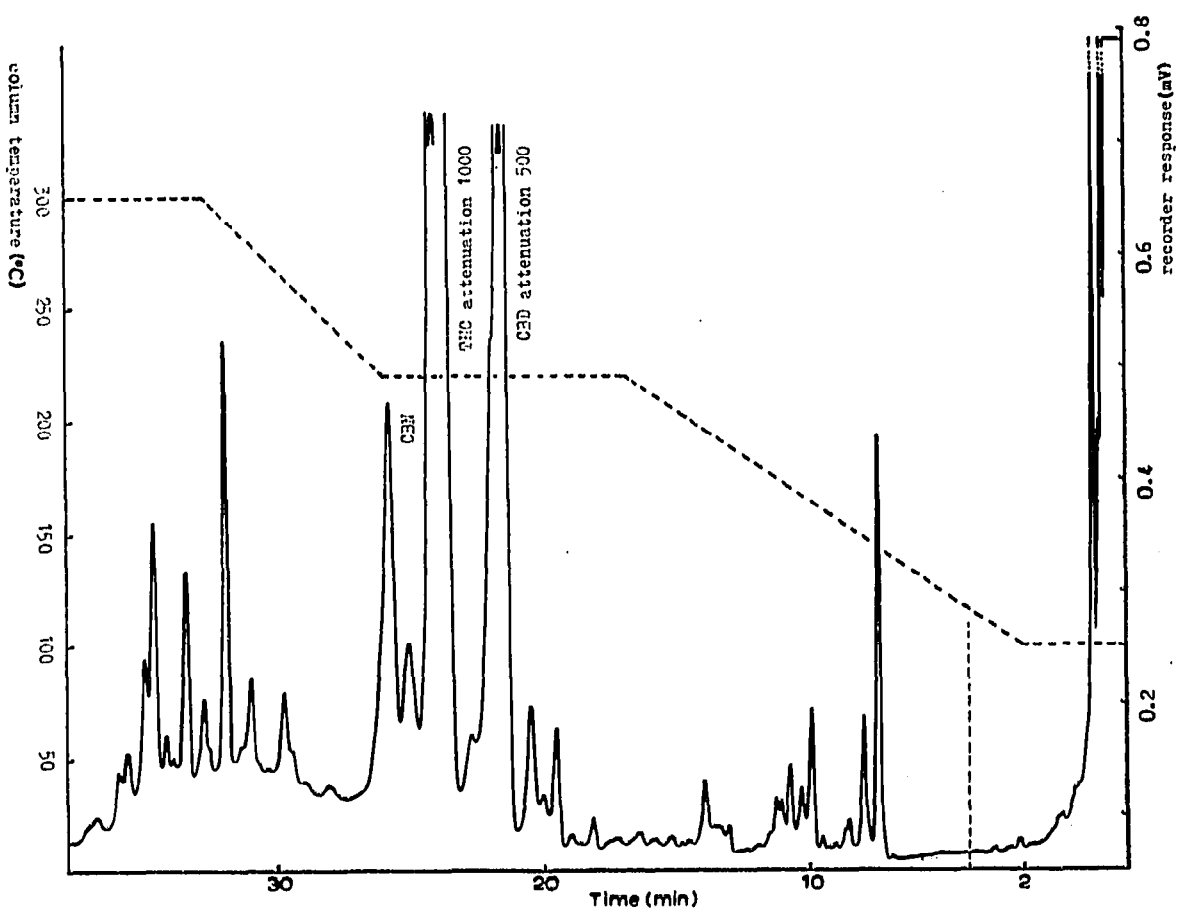

Fig. 1. Gas chromatogram of a hashish extract. The broken dashed line shows the column temperature: 0–2 min at 100°, 2–17 min at 8°/min, 17–26 min at 220°, 26–32.7 min at 12°/min, 32.7–38 min at 300°. The vertical dashed line shows the retention time (about 4 min) of methyl nonanoate. Attenuation: × 200.

there is a non-geographical factor which may dominate the chemical composition of the cannabis resin.

Apart from the geographical origin, a question of great forensic interest is whether cannabis samples seized in different places can be assigned to a common lot. Sometimes conclusions may be drawn from the shape of the material, *e.g.* surfaces of fracture on hashish lumps. In other cases, the only way to tackle the problem is by a comparison of the chemical compositions. Detailed pictures of these may be obtained by gas chromatographic analysis of the extractable part of the cannabis material. A suitable technique for such analyses was described in previous publications from this laboratory[6,7].

Experimental

Apparatus. The gas chromatograph used was a Perkin-Elmer F11 with a No.4 analyser unit (all glass, dual-channel system), linear temperature programmer, flow control unit and a Perkin-Elmer 165 potentiometric recorder.

Columns were of O.D. 6 mm (0.25 in) and I.D. 2 mm glass tubes of 1.9-m length and 130-mm coil diameter, packed with Chromosorb W AW-HMDS (80–100 mesh), coated with 5 % JXR methyl silicone.

Other conditions were: carrier flow, 30 ml of nitrogen per minute; injector

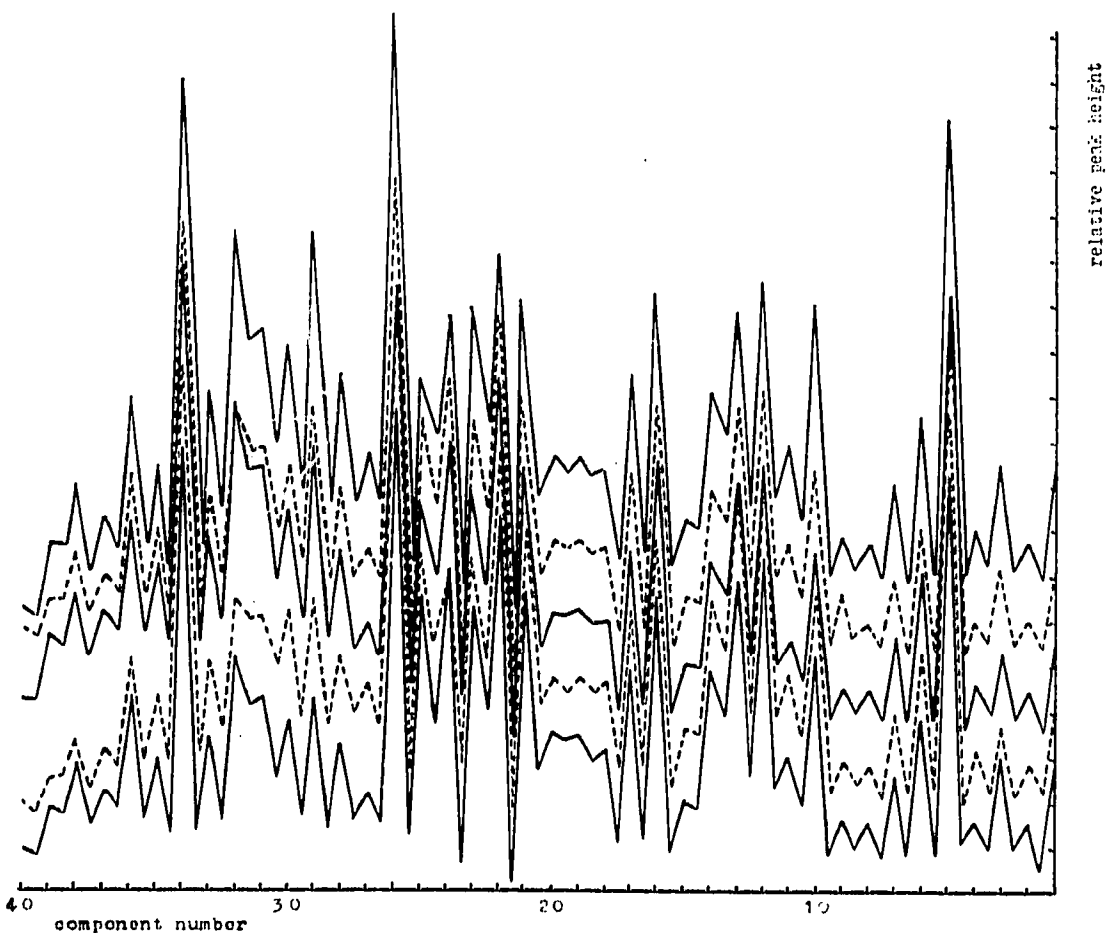

Fig. 2. Comparative analysis of 5 hashish samples, A_1-A_5, taken from different places in the same lot, A. The curves are simplified versions of the original chromatograms and are displaced on the y-axis by arbitrary increments.

temperature, 225°; column temperature program, according to Fig. 1; hydrogen inlet pressure, 1.3 atm; air inlet pressure, 2.0 atm.

Procedure. The hashish samples investigated had been seized by the Swedish police or customs authorities and were of unknown geographical origin. After grinding to a fine powder, 1 g of each sample was extracted in a Soxhlet apparatus with methylene chloride for 6 h. The extracts were made up to 2 ml and 1 μl was injected into the gas chromatograph.

Results and discussion

A typical gas chromatogram is shown in Fig. 1. The heights of the peaks at corresponding retention times were compared in the diagrams. In several cases the chemical composition within the same lot of hashish showed very small variations. A typical example is shown in Fig. 2.

It is known that the composition of the resin in different parts of the same plant and in corresponding parts of different plants within the same cultivation show considerable variations. The smallness of the variations within a lot examined and shown in Fig. 2 is probably the result of homogenizing (*e.g.* grinding) the material. As the comparison includes some 40 components, the probability of such a correlation being a coincidence is very small. Thus, one can say that the gas chromatographic

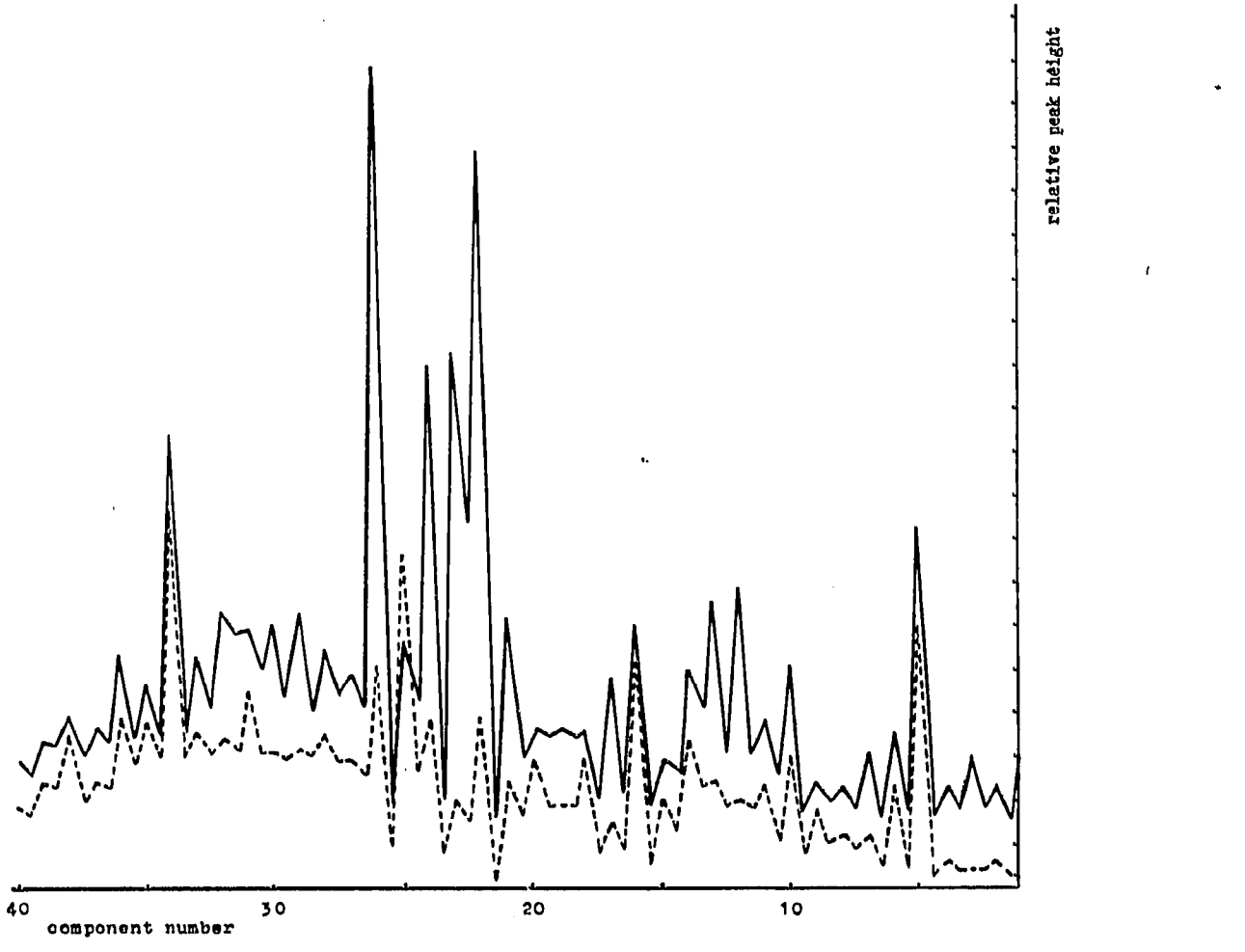

Fig. 3. Comparative analysis of 2 hashish samples taken from the different lots, *A* and *B*.

analysis gives a detailed description of the *average* composition of the lot. It seems very unlikely that samples of different origin would give such closely comparable results.

A comparison of the gas chromatograms from two pairs of different hashish samples (Figs. 3, 4) supports this assumption. These samples also differed from each other in appearance, both in shape and colour.

Summing up, if two or more hashish samples are quantitatively analyzed with regard to some 40 components, and the chromatograms are found to be in accordance, such as shown in Fig. 2, it may be assumed that they originate from a common larger lot with a homogenous composition. Fig. 5 shows a case of a comparative analysis of 4 hashish samples which confirmed the suspicion of the police that they originated from the same lot.

If two samples, X and Y, are compared in respect to the amounts of the 40 components and the corresponding peak heights of the ith component are denoted h_i^X and h_i^Y, respectively, the correlation may be expressed by:

$$d_{XY} = \left(\sum_{i=1}^{40} (h_i^X - h_i^Y)^2\right)^{\frac{1}{2}} \qquad (1)$$

For the comparison to be accurate, an internal standard should be used. Methyl nonanoate was found suitable for this purpose. Its retention time is shown in Fig. 1.

To study the variations within a lot, A, 40 mean values were calculated from the peak heights of the samples A_1–A_5 (Fig. 2). Five d values were then obtained from the above formula setting X equal to $\bar{A}$ (the hypothetical mean sample) and Y

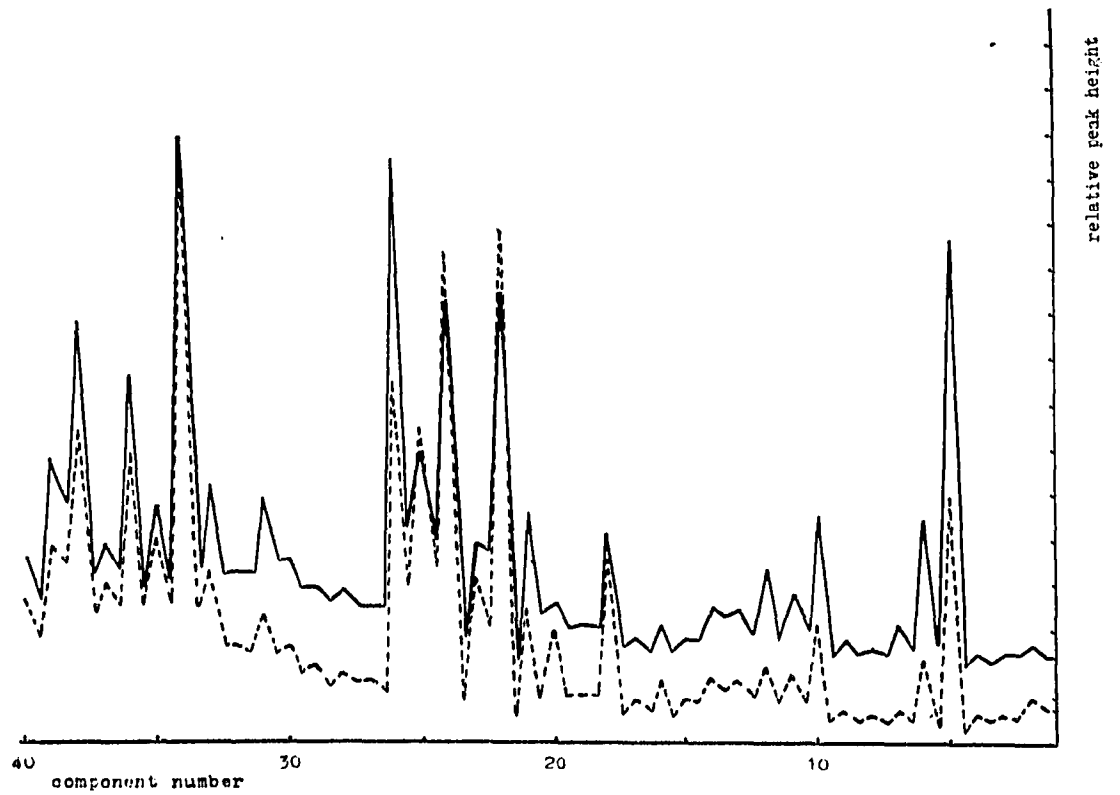

Fig. 4. Comparative analysis of 2 hashish samples taken from the different lots, C and D.

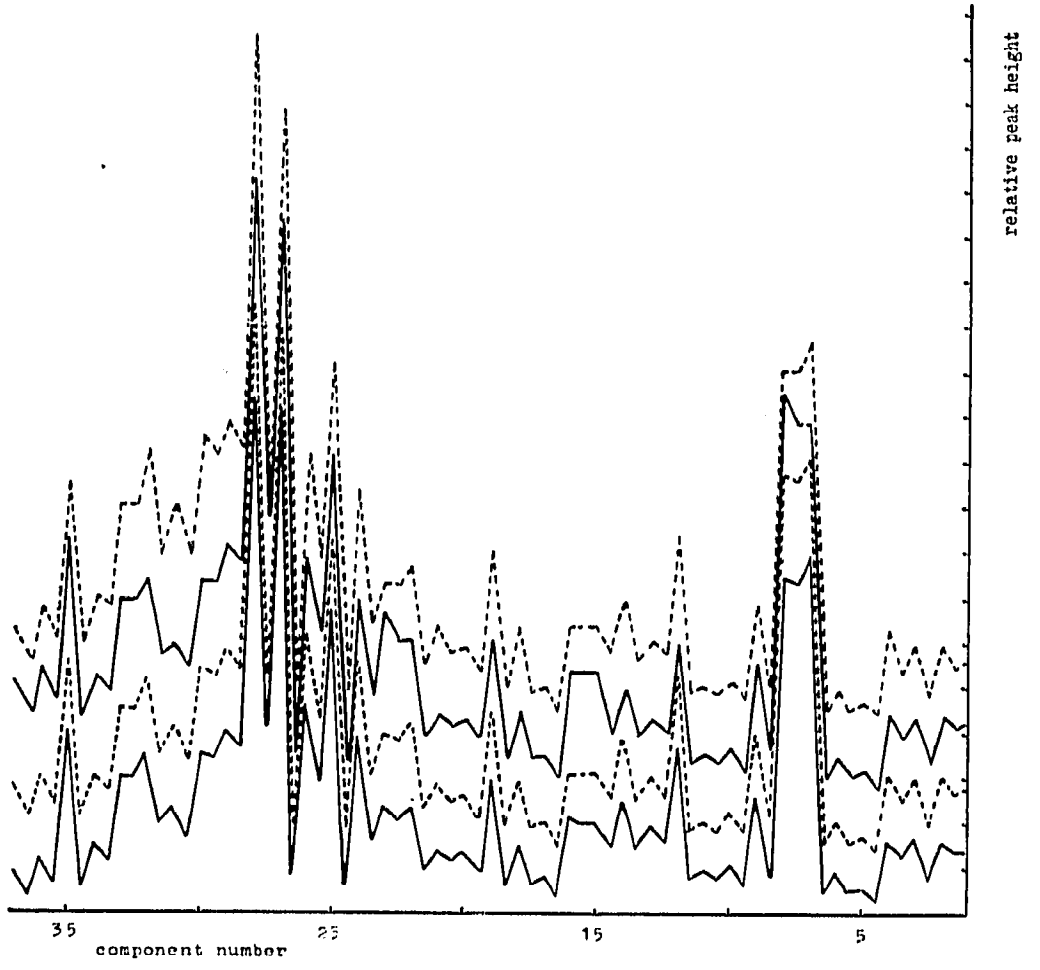

Fig. 5. Comparative analysis of 4 seized hashish samples suspected to originate from the same lot.

TABLE I

COMPARISON OF d VALUES WITHIN THE SAME LOT, A, AND BETWEEN 2 DIFFERENT LOTS, A AND C

$d_{\bar{A}A_1}$	$d_{\bar{A}A_2}$	$d_{\bar{A}A_3}$	$d_{\bar{A}A_4}$	$d_{\bar{A}A_5}$	$d_{\bar{A}C}$
8	6	4	10	8	64

equal to A_1, A_2, ..., A_5, respectively. The greatest deviation was obtained for A_4. This may be explained by the fact that A_4 was taken from the surface layer of A and thus may have been somewhat changed by evaporation or air oxidation. In order to study the variation between different lots of hashish, a sixth d value was accordingly calculated from $\bar{A}$ and C (Fig. 4). The six d values are listed in Table I.

Possibly the comparative method described here can be applied to other narcotics, *e.g.* opium, provided that the variation in the composition between different lots is of greater order of magnitude than within the same lot and that the comparison includes a sufficient number of components.

The author wants to thank Dr. ANDREAS MAEHLY, Director of the National Laboratory of Forensic Science, for his interest and encouragement.

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Received February 10th, 1972