CHROM. 5973

Minor components of cannabis resin

II. Separation by gas chromatography, mass spectra and molecular weights of some components with shorter retention times than cannabidiol

In a previous paper[1] the separation of some 40 minor components from a hashish extract by gas chromatography was reported. The protolytic properties of these components were studied. About 25 of them, hereinafter called the light components, had shorter retention times than cannabidiol. With few exceptions they turned out to lack pronounced acidic or basic properties. This observation is in accordance with earlier investigations, showing that many of these components are terpenic hydrocarbons and alcohols[2] and homologues of the almost neutral main components, cannabidiol, tetrahydrocannabinol and cannabinol[3,4].

Up until now, little attention has been paid in the literature to the heavy minor components eluting after cannabinol. The column bleeding, which so far made the mass spectrometric analysis of these components very difficult, has now been significantly reduced by the use of a recently introduced stationary phase.

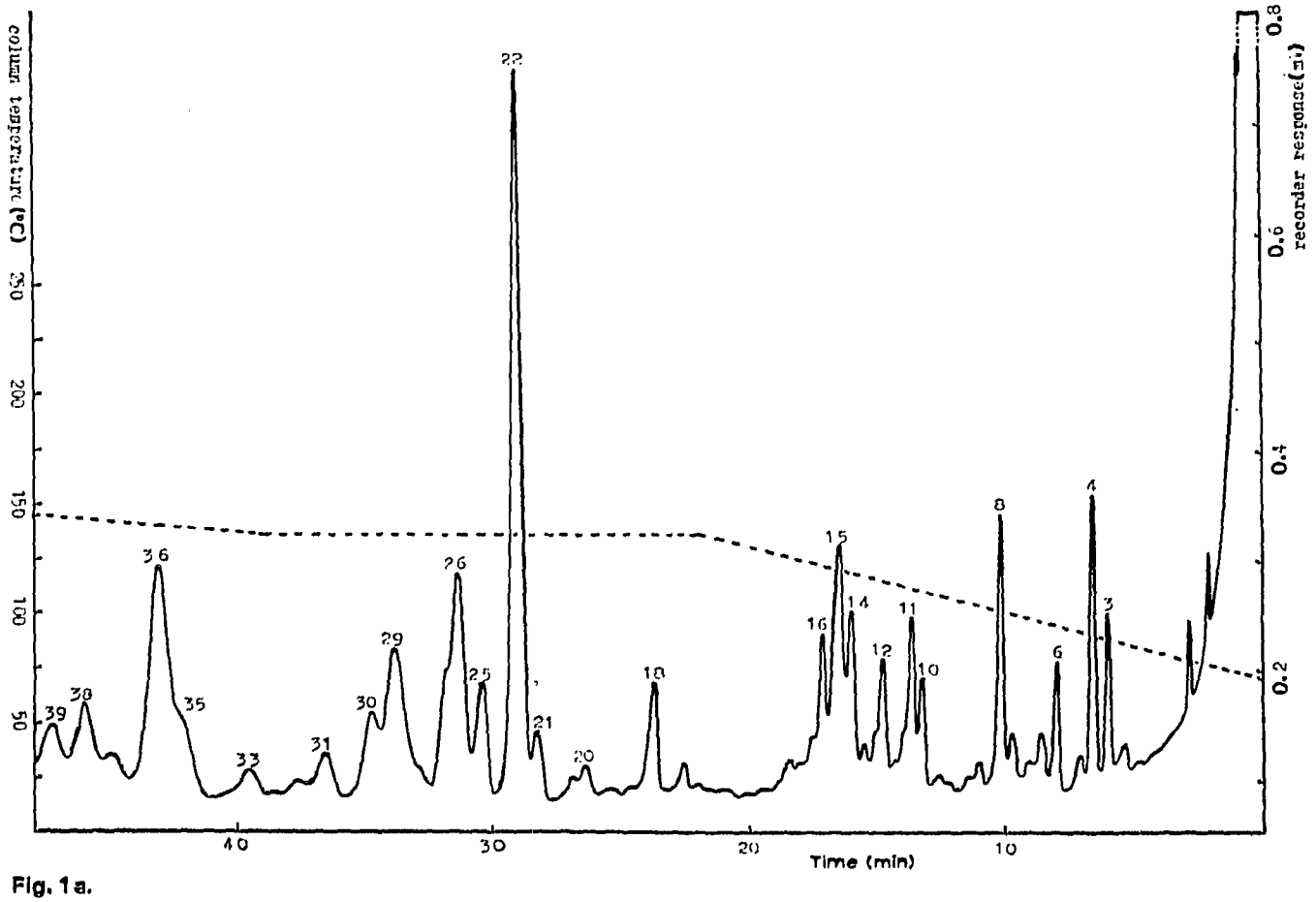

Fig. 1a.

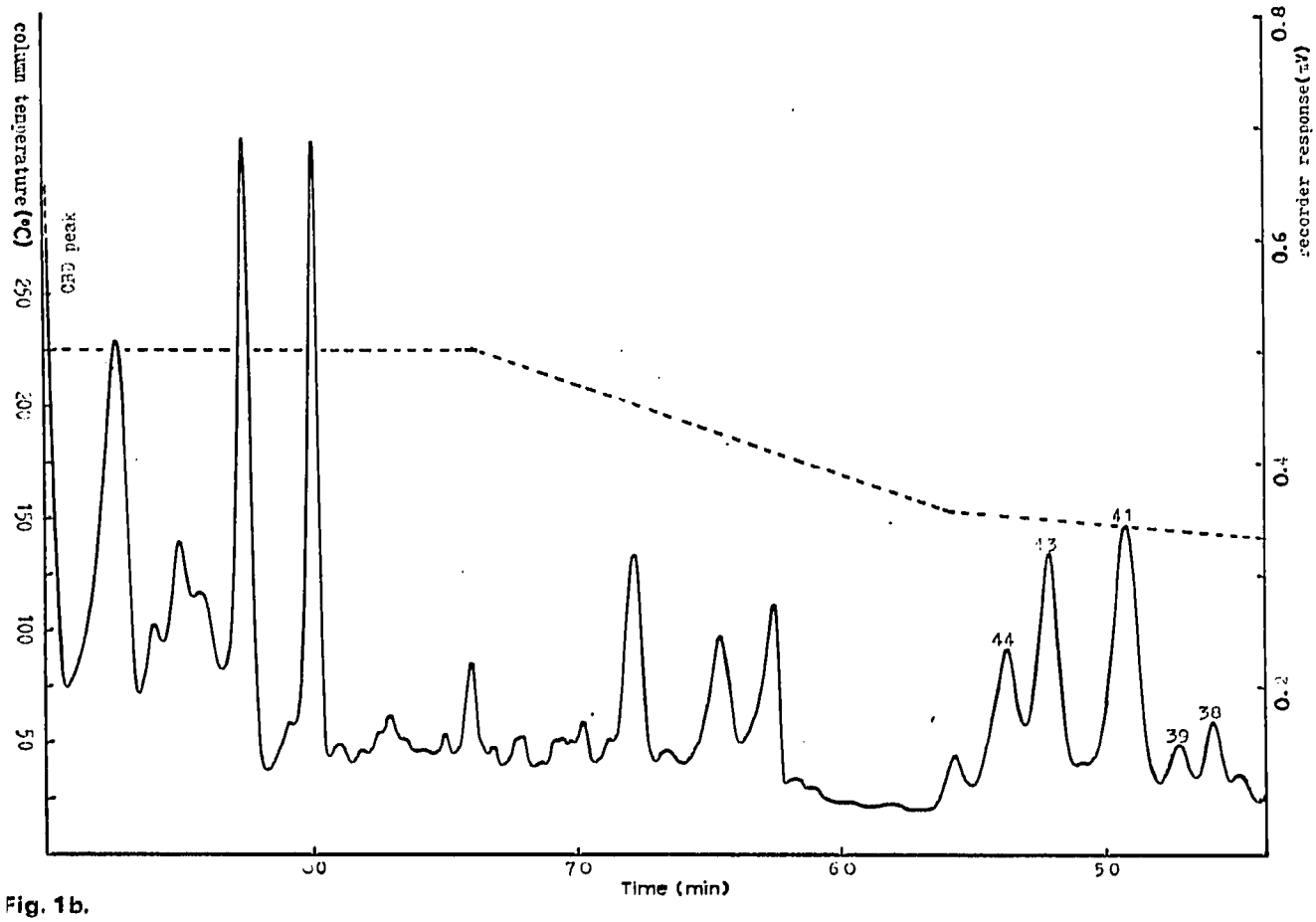

Fig. 1b.

Fig. 1. (a) and (b). Gas chromatogram of the light fraction of the hashish extract. The broken dashed line shows the column temperature: start at 70°, 0–22 min at 3°/min, 22–39 min at 136°, 39–56 min at 1°/min, 56–74 min at 4°/min, 74–90 min at 225°. Mass spectra have been recorded for the numbered peaks. Attenuation: × 200.

Experimental

Apparatus. The gas chromatograph used was a Perkin-Elmer F11 with a No. 4 analyser unit (all glass system), linear temperature programmer, flow control unit and a Perkin-Elmer 165 potentiometric recorder. The combination instrument used for gas chromatography–mass spectrometry was an LKB 9000. An IBM 1800 was used for the calculations.

Columns. For the light fraction the column was an O.D. 6 mm (0.25 in.), I.D. 2 mm glass tube of 4.5 m length and 100 mm coil diameter, packed with Gas-Chrom Q (60–80 mesh), coated with 3 % OV-101 methyl silicone.

For the heavy fraction the column was a O.D. 6 mm (0.25 in.), I.D. 2 mm glass tube of 2.7 m length and 130 mm coil diameter, packed with Gas-Chrom Q (60–80 mesh), coated with 5 % Dexsil 300 GC polycarborane–siloxane.

Procedure. To reduce the relatively expensive running time of the LKB 9000 instrument, the separation technique was developed on the F11 chromatograph. This is run with nitrogen carrier gas at a constant flow of 30 ml/min, whereas in the LKB 9000 instrument helium is used at a constant inlet pressure of 3.0 atm. Therefore the original column temperature program had to be slightly modified.

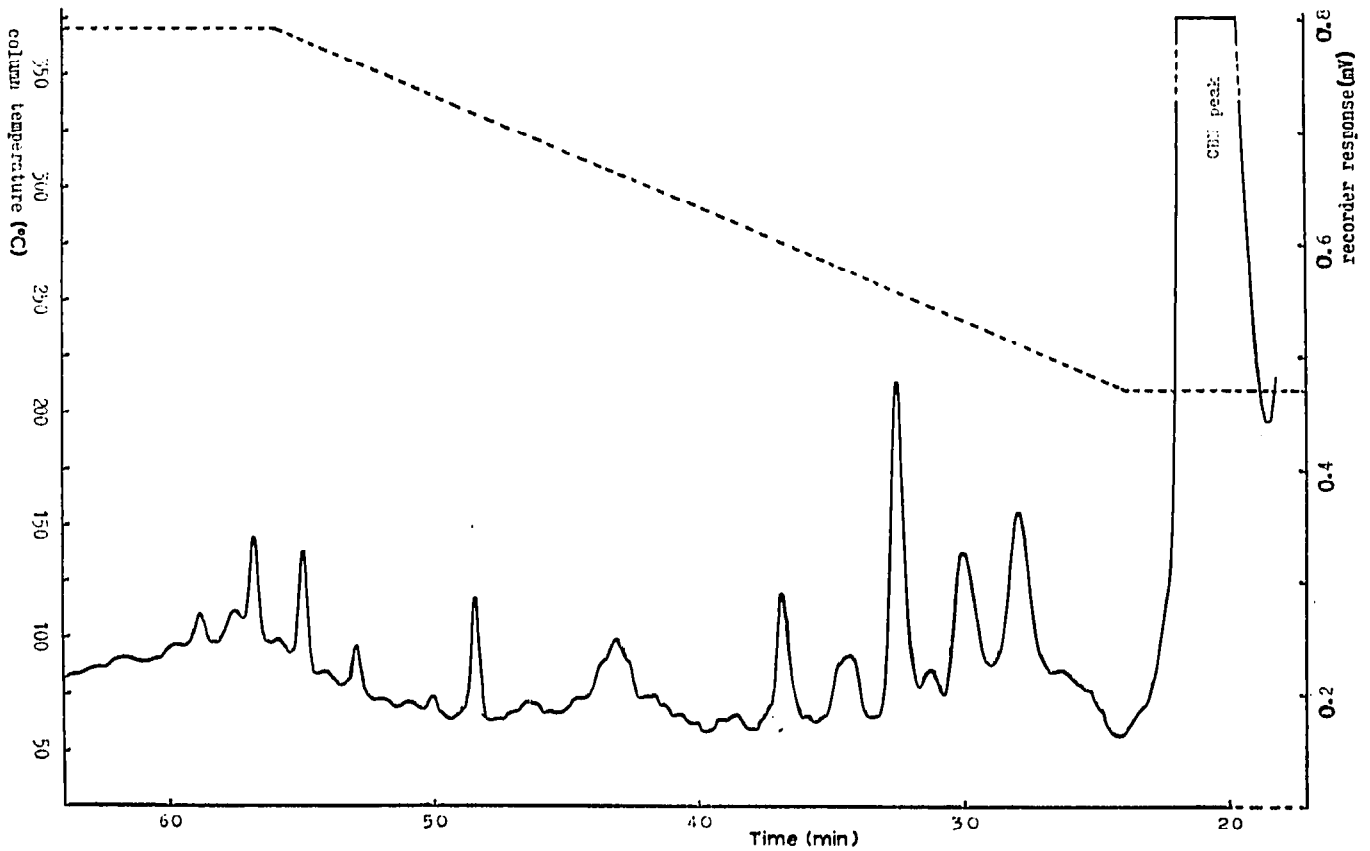

Fig. 2. Gas chromatogram of the heavy fraction of the hashish extract. The broken dashed line shows the column temperature: 0–24 min at 210°, 24–56 min at 5°/min, 56–64 min at 370°. Attenuation: × 1000.

Three grams of hashish (the same material as previously investigated[1]) was extracted in a Soxhlet apparatus with methylene chloride for 6 h. The extract was made up to 5 ml. 1 μl of this extract was injected into the F11 chromatograph. The injector temperature was 225°. The column temperature programs are shown in Figs. 1 and 2.

For the purpose of mass spectrometry about 5 μl of the extract were injected

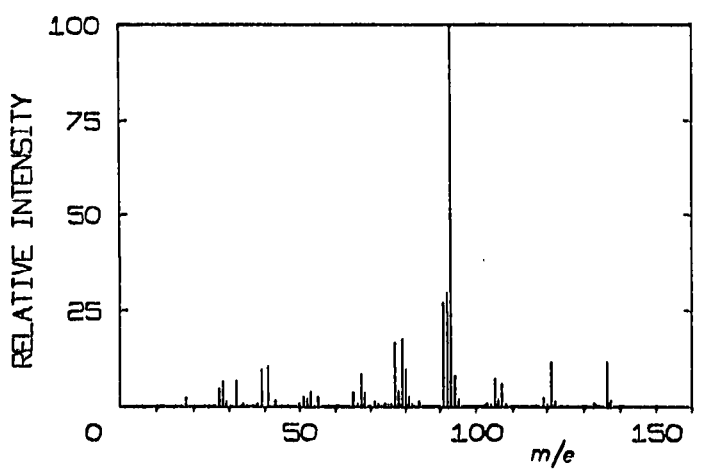

Fig. 3. Mass spectrum of peak No.4.

into the LKB 9000 instrument. Except for the modifications mentioned above, the gas chromatographic conditions were the same as for the F11 chromatograph. The electron energy was 70 eV and the ion source temperature was about 270°. By means of a data acquisition system (continous off-line recording) mass spectra were recorded for the peaks numbered in Fig. 1. The spectra were corrected for the background, normalized and drawn by the computer.

Results and discussion

The separation of the light components previously reported[1] was improved by using glass columns of 4.5-m length as described above. Fig. 1 shows a gas chromatogram of the light fraction. Some 60 peaks can now easily be observed, but the separation is not complete. Probably an appreciable improvement can be achieved only with capillary columns. Experiments with such columns are in progress.

Mass spectra of the components of the light and heavy fractions will be interpreted and discussed in a forthcoming publication[5]. Up to now spectra have been recorded for the peaks numbered in Fig. 1. Corresponding probable molecular weights are listed in Table I. Four of the mass spectra are shown in Figs. 3–6.

A gas chromatogram of the heavy fraction is shown in Fig. 2. A detailed mass spectrometric analysis may reveal whether the components eluting in this region

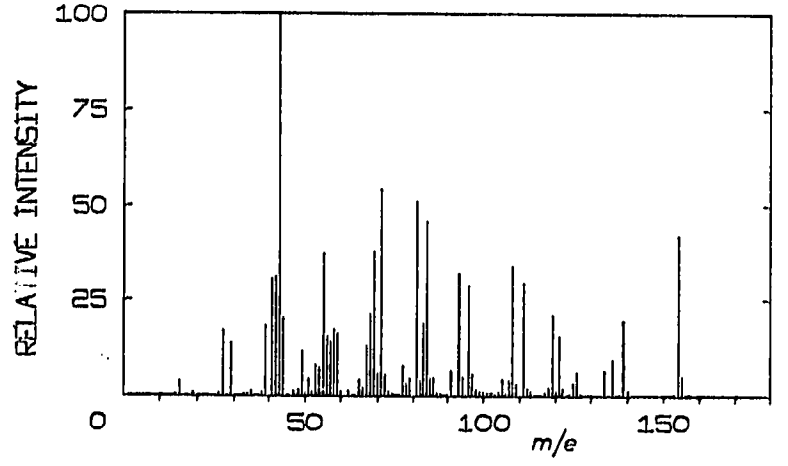

Fig. 4. Mass spectrum of peak No. 8.

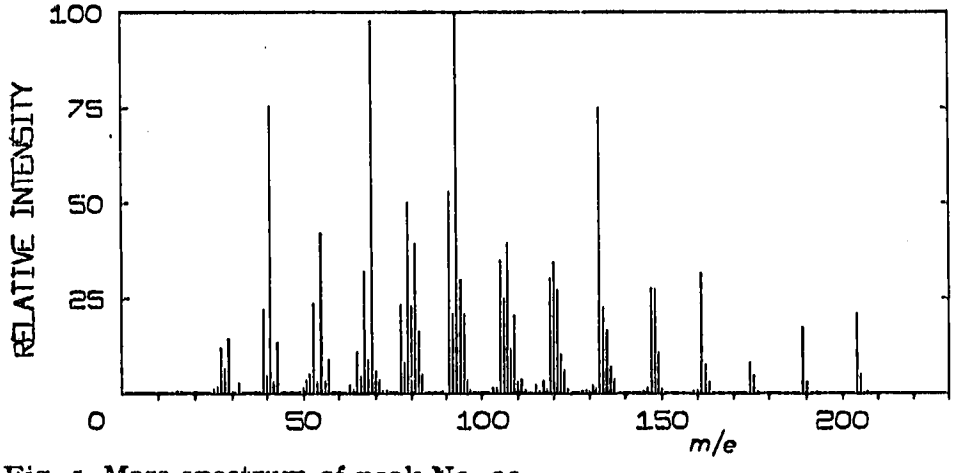

Fig. 5. Mass spectrum of peak No. 22.

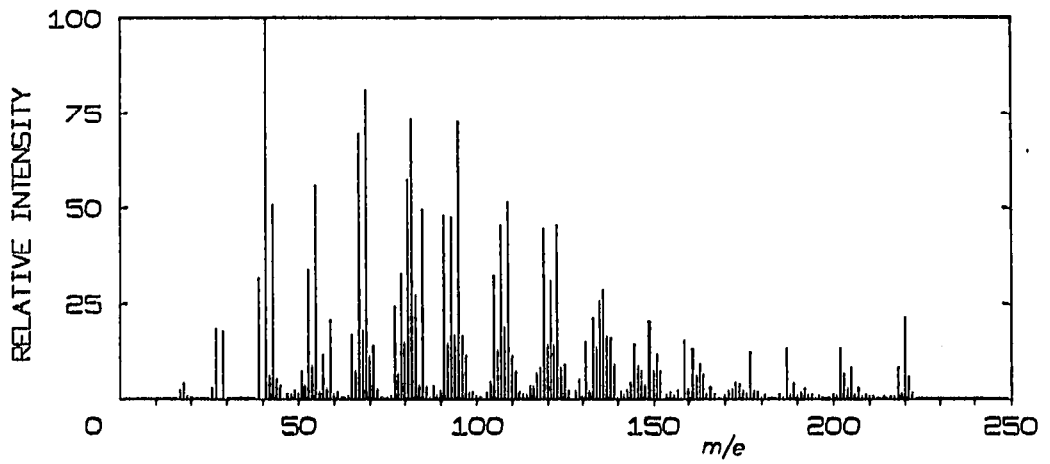

Fig. 6. Mass spectrum of peak No. 41.

TABLE I

PROBABLE MOLECULAR WEIGHTS OF THE COMPOUNDS OF THE LIGHT FRACTION

Peak No. in Fig. 1	*Mol. wt.*	*Peak No. in Fig. 1*	*Mol. wt.*	*Peak No. in Fig. 1*	*Mol. wt.*
3	136	16	154	31	204
4	136	18	150	33	204
6	136	20	204	35	220
8	154	21	204	36	220
10	154	22	204	38	220
11	154	25	204	39	222
12	152	26	204	41	220
14	154	29	204	43	220
15	154	30	204	44	220

are structurally related to the main components of cannabis resin or are compounds of a different nature.

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1 L. STRÖMBERG, *J. Chromatogr.*, 63 (1971) 391.
2 M. C. NIGAM, K. L. HANDA, I. C. NIGAM AND L. LEVI, *Can. J. Chem.*, 43 (1965) 3372.
3 T. B. VREE, D. D. BREIMER, C. A. M. VAN GINNEKEN, J. M. VAN ROSSUM, R. A. DE ZEEUW AND A. H. WITTE, *Clin. Chim. Acta*, 34 (1971) 365.
4 F. W. H. M. MERKUS, *Nature*, 232 (1971) 579.
5 A. MAEHLY, Y. MÅRDE, R. RYHAGE AND L. STRÖMBERG, to be published.

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