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MINOR COMPONENTS OF CANNABIS RESIN

IV. MASS SPECTROMETRIC DATA AND GAS CHROMATOGRAPHIC RETENTION TIMES OF TERPENIC COMPONENTS WITH RETENTION TIMES SHORTER THAN THAT OF CANNABIDIOL

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SUMMARY

Minor terpenic components of cannabis resin were analyzed by gas chromatography and mass spectrometry. Eleven components were tentatively identified by comparing the mass spectra obtained with the known spectra of 134 terpenic compounds. Some of these identifications were supported by comparing the retention data obtained with the corresponding data from the literature.

INTRODUCTION

In Part II[1] of this series, the analysis of minor terpenic components of a hashish material by combined gas chromatography–mass spectrometry (GC–MS) was described. Typical molecular weights of these components were 136, 154, 204, 220 and 222. Earlier investigations by Nigam *et al.*[2] and Bercht *et al.*[3] and recent work by Hood *et al.*[4] showed the presence of monoterpenes, monoterpene alcohols, sesquiterpenes and a sesquiterpene oxide in cannabis. In this paper, MS data and GC retention times of cannabis components of the type described are reported and discussed.

EXPERIMENTAL

The experimental conditions for the GC–MS analysis of minor terpenic components were described in Part II[1]. For the study of retention times, the gas chromatograph used was a Perkin-Elmer F11 with a No. 4 analyzer unit (all-glass system and flame ionization detector). The column was a 1.9-m glass tube of O.D. 6 mm (0.25 in.) and I.D. 2 mm with a coil diameter of 130 mm, packed with Gas-Chrom Q (80–100 mesh), coated with 6% OV-101 methyl silicone. The injection temperature was 220° and the flow-rate of carrier gas (helium) was about 60 ml/min.

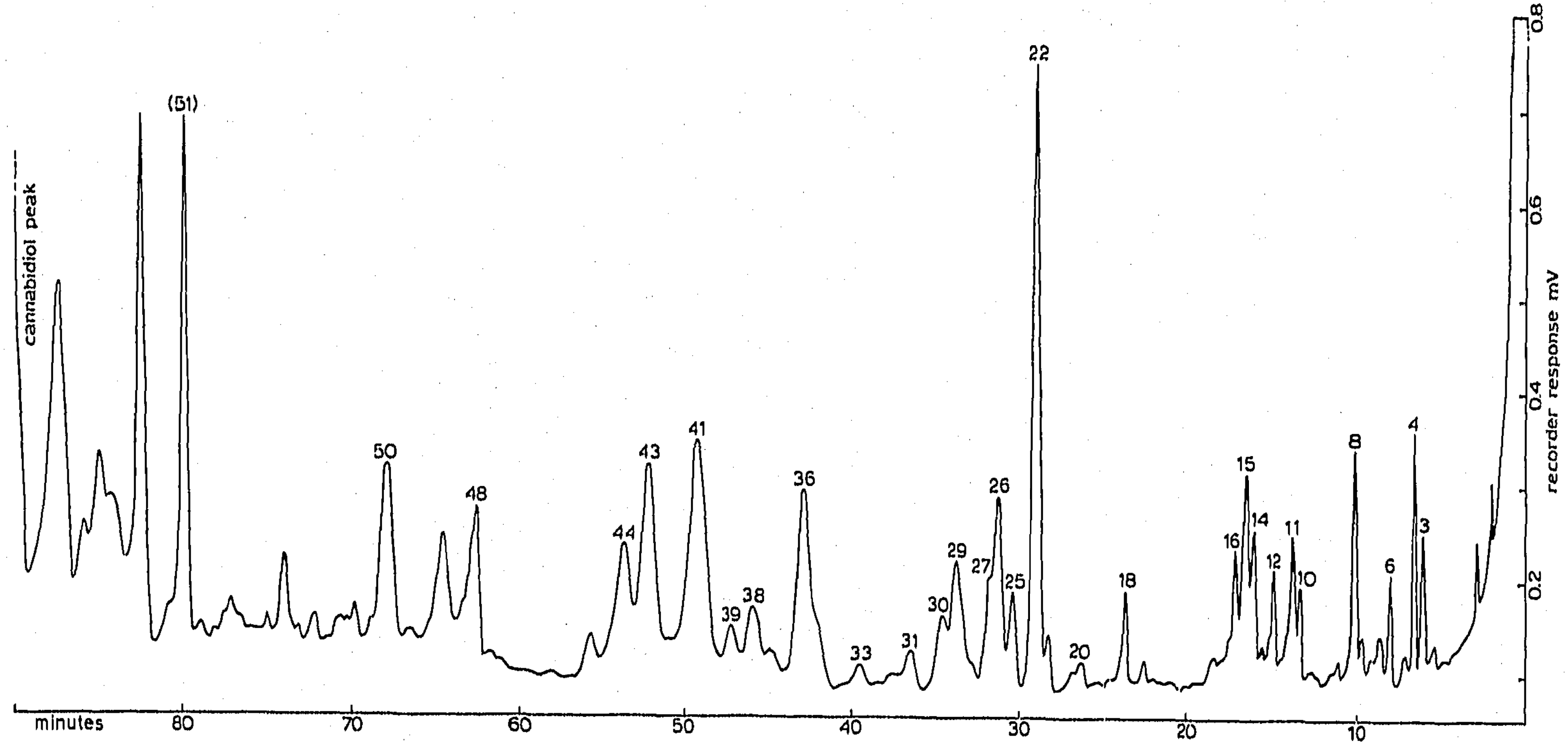

Fig. 1. Gas chromatogram of minor cannabis components with retention times shorter than that of cannabidiol. Mass spectrometric data and relative retention times are reported for the fractions numbered in the chromatogram. Omitted numbers correspond to fractions appearing in gas chromatograms of other cannabis extracts.

RESULTS AND DISCUSSION

Mass spectrometric data

The MS data were obtained by scanning the 28 fractions corresponding to the numbered GC peaks in Fig. 1. This chromatogram was obtained from a regular gas chromatograph with a flame ionization detector and, with few exceptions, coincides with the total ionization current chromatogram obtained in the combined GC–MS instrument, using the same column under slightly modified operating conditions. Three of the peaks did not show distinct fragmentation patterns owing to a low signal-to-noise ratio (fraction 31) or insufficient chromatographic stability (fractions 48 and 50). After noting the *m/e* values of the parent ions, these spectra were excluded from further interpretation. The remaining 25 spectra, shown in Figs. 2–26, were then compared visually with standard spectra of 134 terpenic compounds obtained from the literature[5–11].

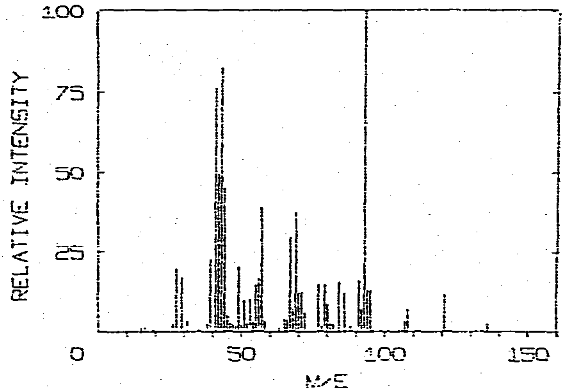

Fig. 2. Mass spectrum of fraction 3.

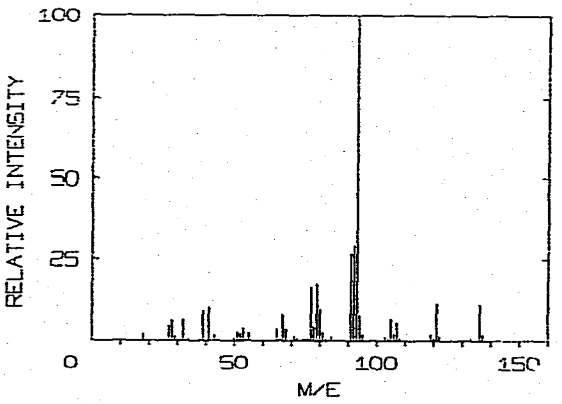

Fig. 3. Mass spectrum of fraction 4.

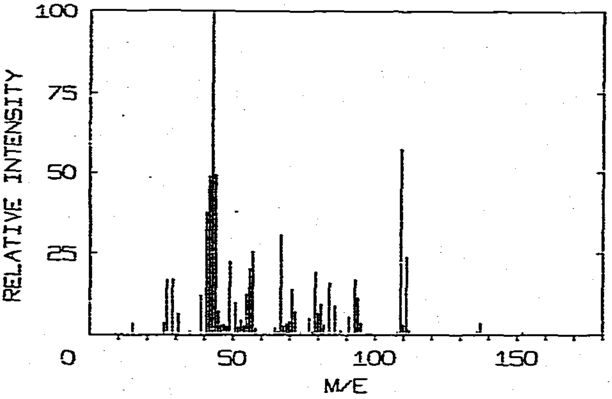

Fig. 4. Mass spectrum of fraction 6.

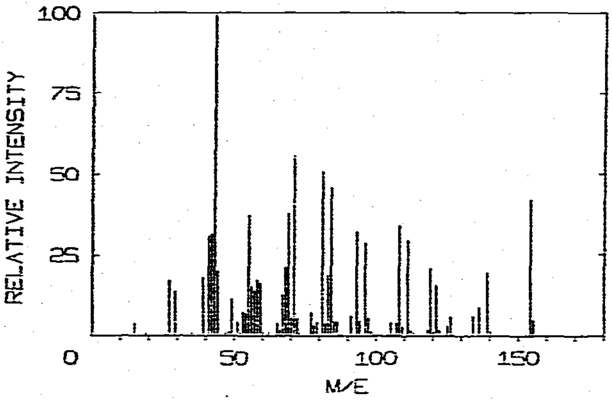

Fig. 5. Mass spectrum of fraction 8.

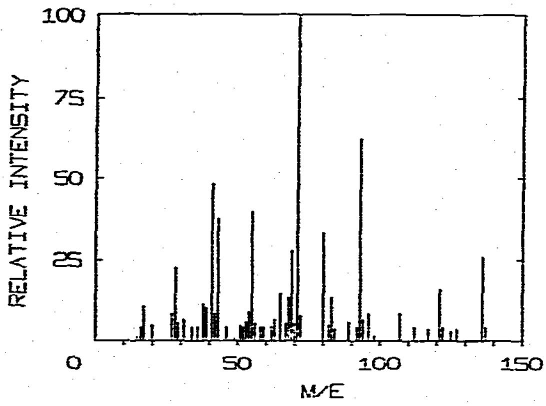

Fig. 6. Mass spectrum of fraction 10.

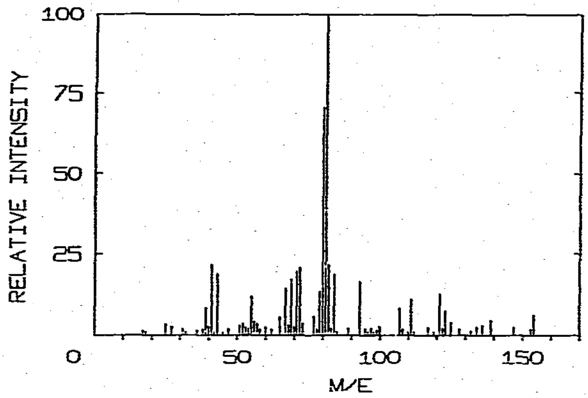

Fig. 7. Mass spectrum of fraction 11.

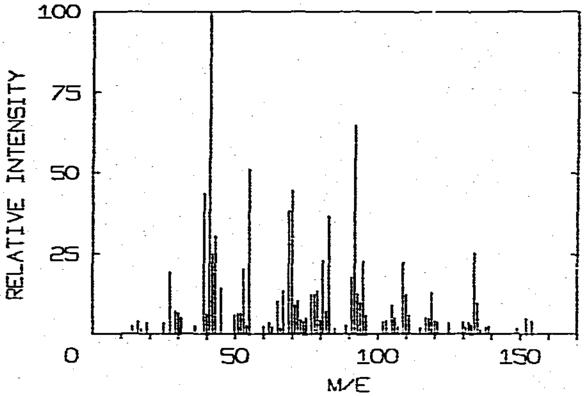

Fig. 8. Mass spectrum of fraction 12.

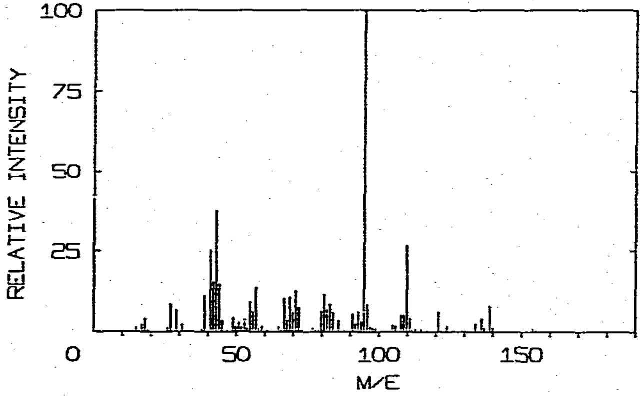

Fig. 9. Mass spectrum of fraction 14.

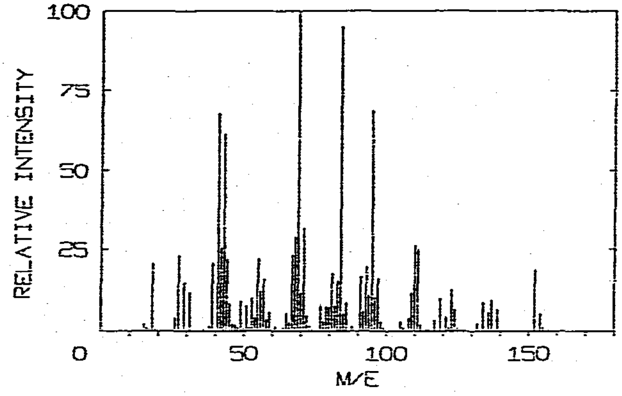

Fig. 10. Mass spectrum of fraction 15.

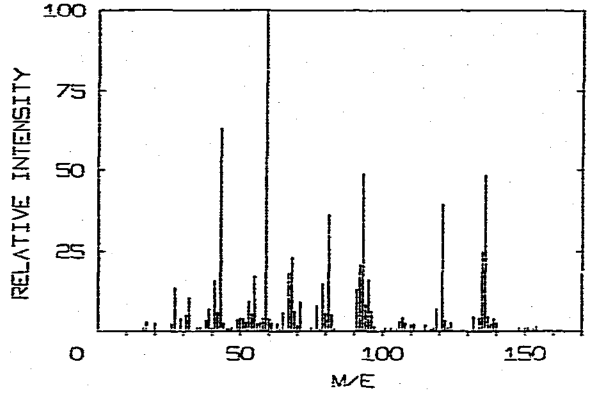

Fig. 11. Mass spectrum of fraction 16.

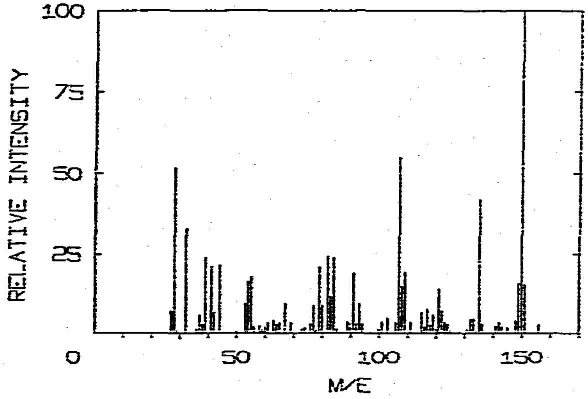

Fig. 12. Mass spectrum of fraction 18.

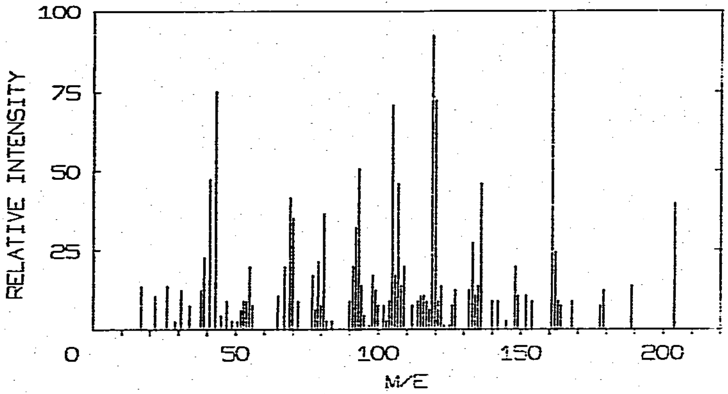

Fig. 13. Mass spectrum of fraction 20.

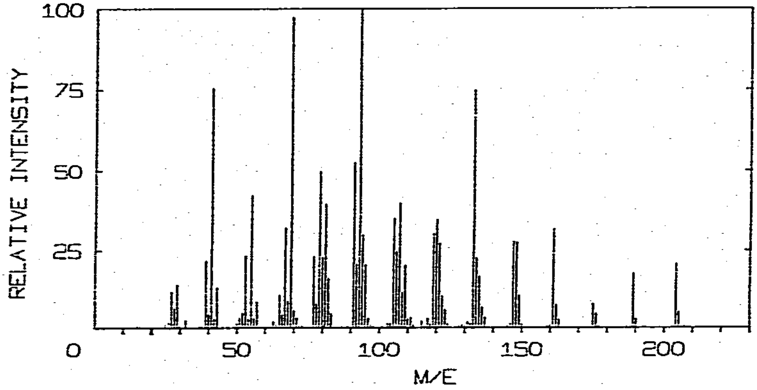

Fig. 14. Mass spectrum of fraction 22.

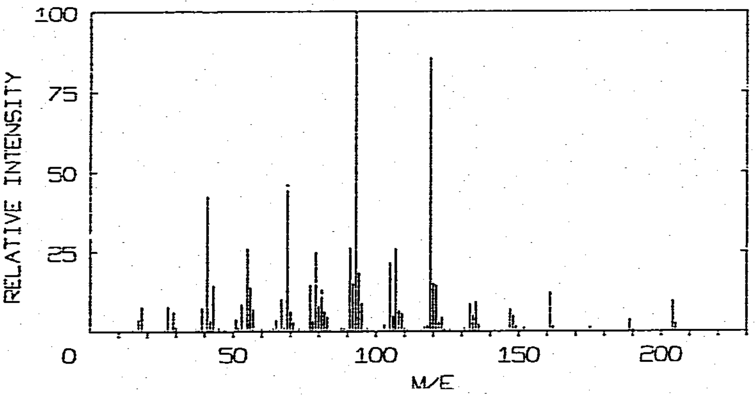

Fig. 15. Mass spectrum of fraction 25.

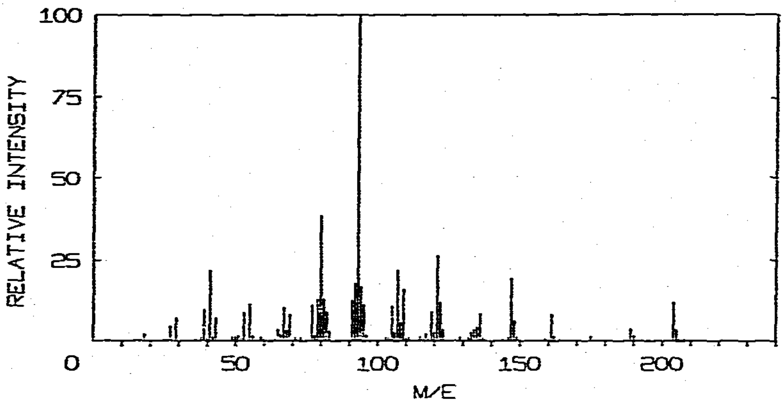

Fig. 16. Mass spectrum of fraction 26.

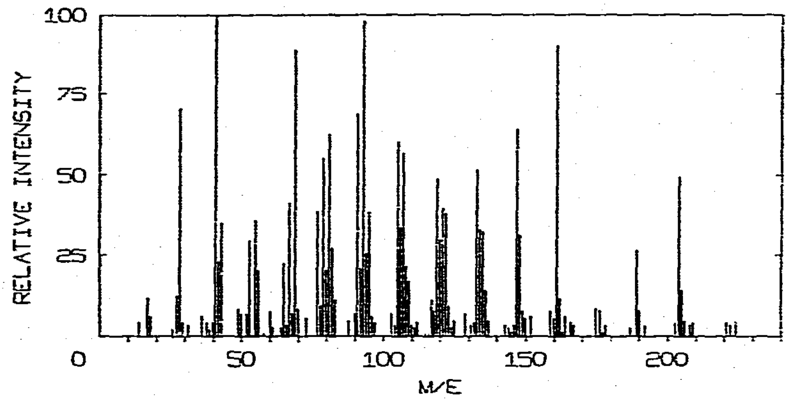

Fig. 17. Mass spectrum of fraction 27.

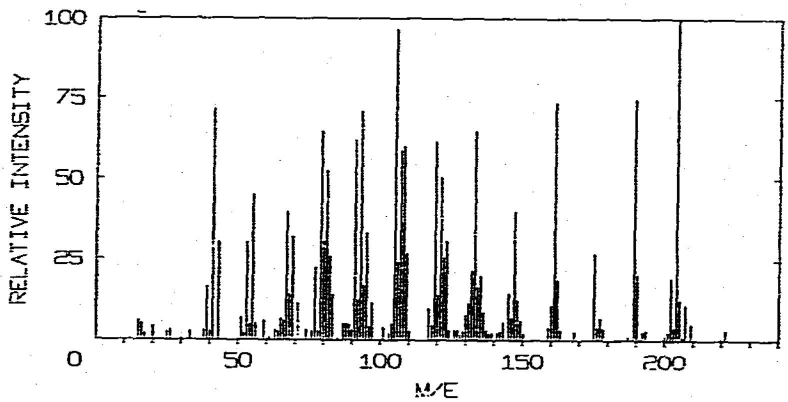

Fig. 18. Mass spectrum of fraction 29.

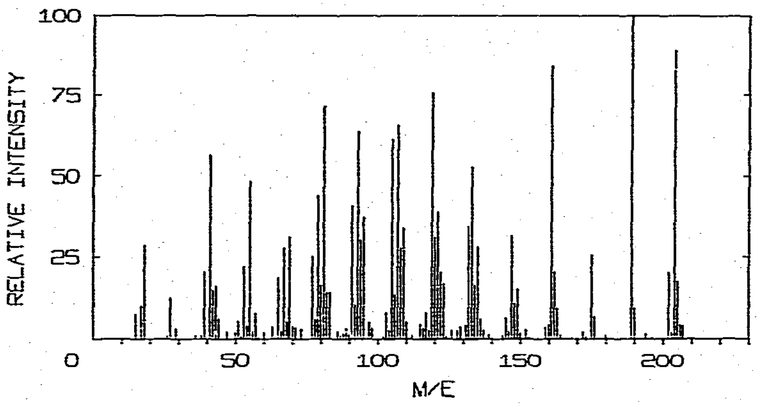

Fig. 19. Mass spectrum of fraction 30.

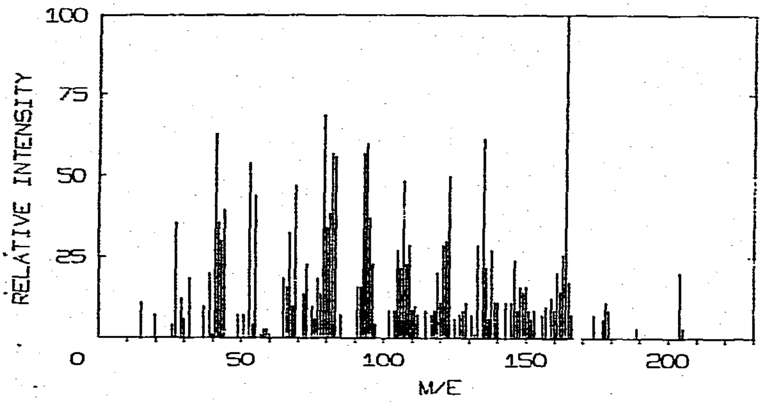

Fig. 20. Mass spectrum of fraction 33.

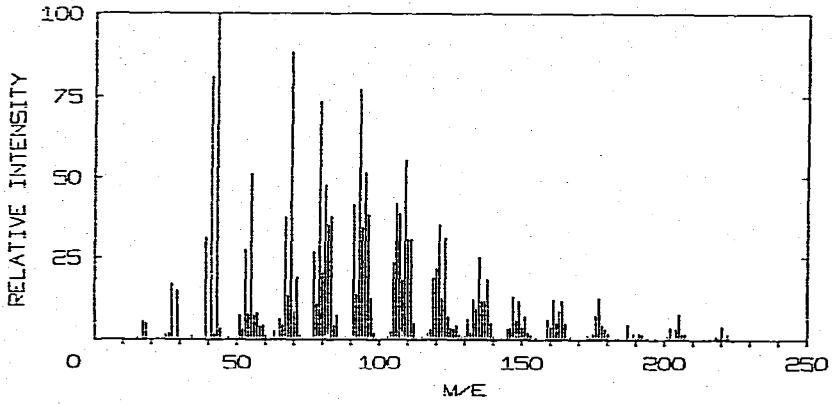

Fig. 21. Mass spectrum of fraction 36.

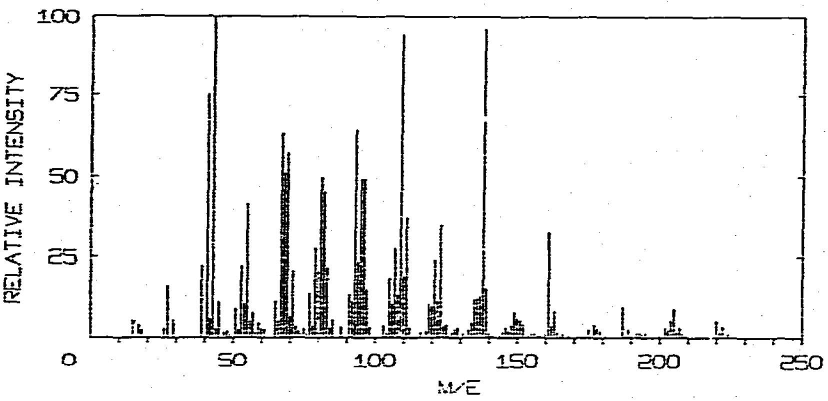

Fig. 22. Mass spectrum of fraction 38.

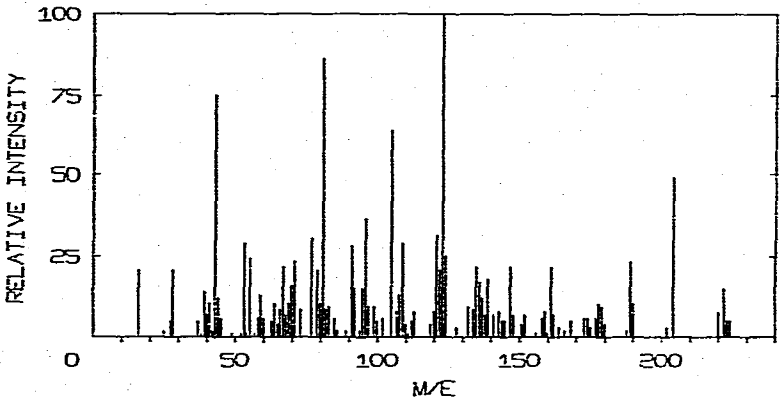

Fig. 23. Mass spectrum of fraction 39.

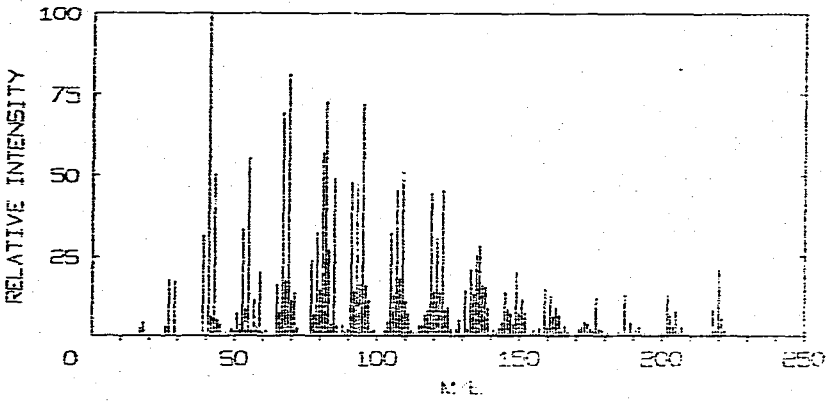

Fig. 24. Mass spectrum of fraction 41.

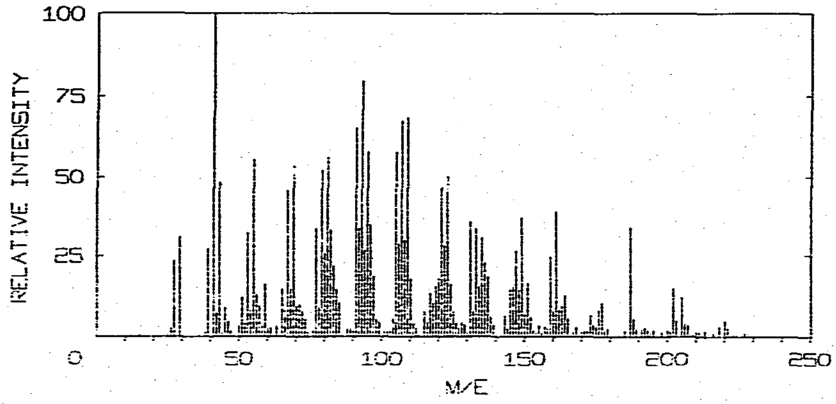

Fig. 25. Mass spectrum of fraction 43.

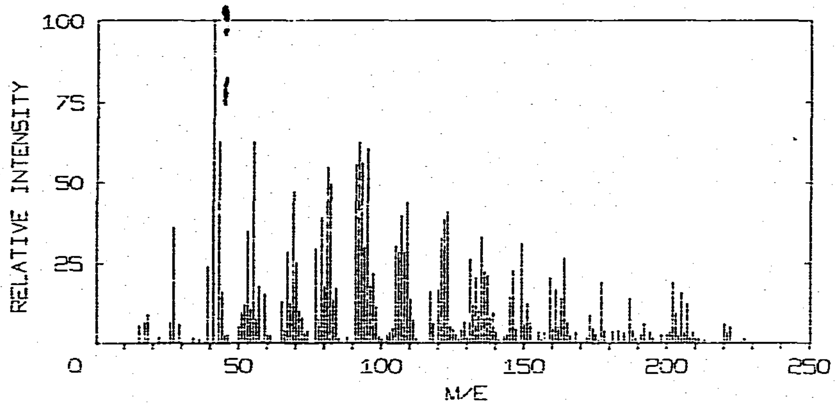

Fig. 26. Mass spectrum of fraction 44.

In nine cases, good agreement was observed between the spectrum of a fraction (4, 10, 11, 16, 22, 25, 26, 27 and 29) and a particular standard spectrum. In two cases, the comparison indicated that the fractions (14 and 18) may be mixtures containing borneol and piperitenone, respectively, as major components. However, the possibility cannot be excluded, of course, that the major components of these two fractions were not among the 134 terpenic compounds under consideration. The comparisons in these eleven cases are shown in Table I. The following comments can be made.

Fraction 10. In Fig. 6, the *m/e* axis ends at 150. However, an extended scan showed a parent peak at *m/e* 154 with an intensity of about 3% of that of the base peak.

Fraction 14. Excessive intensities are observed at *m/e* 41, 42 and 43 (Fig. 9). This cannot be explained by interference from fraction 15 because in that case the intensities at *m/e* 69 and 84 would have been much higher (*cf.*, Fig. 10).

Fraction 18 (Fig. 12). The slight asymmetry of the GC peak as seen in Fig. 1

TABLE I

COMPARISON OF THE MASS SPECTRA OBTAINED IN THIS STUDY WITH CORRESPONDING SPECTRA FROM THE LITERATURE

Experimental intensities are listed in order of decreasing magnitude without omitting any values except those with *m/e* 18, 28, 32, 40 and 44 (H_2O, N_2, O_2, Ar and CO_2, respectively). The ten highest intensities of the standard spectra are printed in bold.

Fraction No. (Fig. 1)	*Standard*	*m/e values* *Relative intensities of the spectra obtained in the study* *Relative intensities of the standard spectra*														
4	α-Pinene	93	92	91	79	77	136	121	80	41	39	94	69			
		100	30	27	18	16	11	11	10	10	9	8	0			
		100	**10**	**20**	**18**	**25**	**12**	9	**10**	**16**	1	**11**	**10**			
10	Linalool	71	93	41	55	43	80	69	136	121	65	68	67			
		100	62	48	40	38	33	28	26	16	14	13	5			
		95	**100**	**57**	**56**	**54**	**40**	**50**	12	**25**	2	**22**	**22**			
11	Fenchyl alcohol	81	80	82	41	72	71	84	43	93	69	111				
		100	71	22	22	21	20	19	19	17	17	12				
		100	**75**	**20**	3	**25**	**20**	**26**	**25**	**20**	**25**	**15**				
14	Borneol	95	43	110	41	42	57	71	81	69	39	139	82	121	93	136
		100	38	27	25	16	14	13	12	11	11	8	7	6	6	4
		100	**5**	**25**	1	0	1	**5**	3	**5**	0	**10**	**5**	**5**	**10**	**5**
16	α-Terpineol	59	43	136	93	121	81	135	68	92	67	79				
		100	63	49	49	39	36	25	23	21	18	15				
		100	**30**	**30**	**60**	**35**	**35**	2	**20**	**20**	**20**	**15**				
18	Piperitenone	150	107	135	82	84	39	79	41	109	91	151	121	108	122	
		100	55	42	25	24	24	21	21	19	19	16	15	15	7	
		100	**45**	**30**	**20**	1	0	9	3	**20**	**10**	**15**	**10**	**15**	**10**	
22	β-Caryophyllene	93	69	133	41	91	79	55	107	81	105					
		100	98	75	75	53	50	42	40	39	35					
		100	**100**	**62**	**52**	**52**	**58**	**39**	**41**	**52**	**47**					
25	*trans*-α-Bergamotene	93	119	69	41	91	107	55	79	105	94	77				
		100	86	47	42	27	26	26	25	22	19	15				
		100	**85**	**50**	**50**	**20**	**25**	**30**	**20**	**20**	14	**20**				
26	Humulene	93	80	121	107	41	147	92	94	109	79					
		100	39	27	22	22	19	18	17	16	13					
		100	**35**	**27**	**14**	**23**	**14**	**17**	**14**	**11**	**14**					
27	Alloaromadendrene	41	93	161	69	91	147	81	105	107	79	133				
		100	99	91	89	69	64	63	60	57	55	52				
		94	**80**	**100**	**71**	**84**	50	**61**	**75**	**72**	**64**	**61**				
29	α-Gurjunene	204	105	189	161	41	93	133	79	119	91	55				
		100	97	75	74	72	71	65	65	62	62	45				
		100	**88**	**79**	**95**	**72**	**40**	**47**	35	**68**	**68**	**47**				

was more pronounced in the combined GC–MS analysis, which supports the assumption that this fraction represents a mixture.

Fractions 26 and 27 (Figs. 16 and 17). These fractions were only partially separated, as seen in Fig. 1. In order to obtain a good separation of the two spectra, fraction 26 was scanned before the peak maximum was reached and fraction 27 was scanned as late as possible with regard to the signal-to-noise ratio.

Fraction 29 (Fig. 18). This fraction was scanned before the peak reached its maximum in order to avoid interference from fraction 30.

The remaining 123 standard compounds under consideration cannot be major constituents of the remaining fourteen fractions, as the major peaks of their mass spectra do not dominate the mass spectra of these fractions. The ten peaks of highest intensity observed in the latter mass spectra are listed in Table II. The following comments can be made.

Fraction 6. Assuming that the peaks at *m/e* 137 and 109 (Fig. 4) correspond to the fragmentation of methyl and propyl groups, respectively, the parent peak is at *m/e* 152. Previous analysis of this fraction did not show this peak and the molecular weight was believed to be 136 (ref. 1).

Fraction 12 (Fig. 8). This fraction was previously considered[1] to be a mixture of *trans*-pinocarveol (mol. wt. 152) and a monoterpene alcohol (mol. wt. 154), and the two peaks at *m/e* 152 and 154, respectively, were believed to be parent peaks. However, it has now become possible to obtain the complete spectra of fractions 15, 36, 38 and 44, which were found to show relatively intense M—2 peaks. Distinct M—2 peaks are also present in the literature spectra of carveol and *neo*-isothujyl alcohol[9]. It therefore seems likely that the parent peak lies at *m/e* 154 and not 152 as previously reported[1].

Fractions 36, 38 and 44 (Figs. 21, 22 and 26). As mentioned above, the M—2 peaks of the spectra of these fractions indicate that the parent peaks lie at *m/e* 222 and not at 220 as reported earlier[1].

Further information concerning the structures of the fractions in Table II can be obtained by a more detailed interpretation of their spectra. For example, mass spectra of monoterpene hydrocarbons were comprehensively discussed by Thomas and Willhalm[12]. However, such an analysis is beyond the scope of this study.

Molecular weights, as derived from the *m/e* values for the presumed parent peaks of the 28 mass spectra obtained in this study, are listed in Table III. It seems likely that a molecular weight of 220 corresponds to sesquiterpene oxides, *i.e.*, of β-caryophyllene[2] and humulene, whereas a molecular weight of 222 corresponds to sesquiterpene alcohols.

Gas chromatographic retention times

As mentioned above, OV-101 methyl silicone columns were used for the separation of the cannabis components. Retention data for cannabis terpenes on such columns were also reported by Hood *et al.*[4]. For comparison, a series of experiments under isothermal conditions was carried out as described under Experimental. The components were easily recognized by comparison with the programmed analysis (Fig. 1) by virtue of their relative peak heights.

By the addition of α-pinene to the hashish extract, it was found that fraction 4 had the same retention time as α-pinene. Next, limonene was added to the extract, in which limonene could not be originally detected. The ratio of the relative retention times of fraction 4, limonene and fraction 10 was then found to be 0.46:1.01:1.84 at a column temperature of about 40°. Further, the ratio of the relative retention times of fractions 22, 25 and 26 was found to be 1.00:1.12:1.19 at 108°. Finally, relative retention times of fractions 10–22 and 44–51 were determined at 84° and 155°, respectively. A comparison of these results with those obtained by Hood *et al.*[4] is

TABLE II

THE TEN HIGHEST INTENSITIES OF THE MASS SPECTRA OBTAINED FOR UNIDENTIFIED FRACTIONS

Intensities with *m/e* 18, 28, 32, 40 and 44 are omitted, as in Table I.

Fraction No. (Fig. 1)	*m/e values* *Relative intensities*									
3	93	43	41	42	57	69	67	39	49	56
	100	83	76	49	39	38	30	23	20	16
6	43	109	42	41	67	57	111	49	56	79
	100	57	49	38	31	26	24	23	21	19
8	43	71	81	84	154	69	55	108	93	42
	100	56	51	46	42	38	37	34	32	31
12	41	92	55	70	39	69	83	43	134	42
	100	64	51	45	44	38	37	31	26	25
15	69	84	95	41	43	71	68	110	111	42
	100	95	69	68	61	32	29	26	25	25
20	161	119	43	120	105	93	41	136	107	69
	100	92	75	72	71	51	48	46	46	42
30	189	204	161	119	81	107	93	105	41	133
	100	89	84	76	72	66	64	61	57	53
33	164	79	41	135	94	93	82	83	53	123
	100	69	63	61	60	57	57	56	54	50
36	43	69	41	93	79	109	95	55	81	106
	100	89	81	77	74	56	52	51	48	43
38	43	138	109	41	93	67	69	68	81	96
	100	96	94	75	64	63	57	51	50	49
39	123	81	43	105	204	96	121	77	109	53
	100	87	75	64	49	37	32	31	29	29
41	41	69	95	82	67	81	55	109	43	85
	100	81	73	73	69	57	56	51	51	49
43	41	93	109	107	91	105	95	81	55	69
	100	80	68	67	65	58	58	56	55	54
44	41	92	55	43	95	93	91	81	69	109
	100	63	63	63	61	56	56	55	47	44

shown in Table III. Fractions 4a, 6a and 8a were not subjected to mass spectrometric analysis as they were present in very low concentrations.

The terpenic components described so far belong to the light minor cannabis components, *i.e.*, those eluted before cannabidiol on methyl silicone columns. To this group of minor cannabis components also belong some alkanes, as demonstrated by De Zeeuw *et al.*[13]. A third group of light minor cannabis components are some homologues and isomers of cannabidiol, tetrahydrocannabinol and cannabinol[14]. Some fractions that appear as peaks with retention times of 80–90 min (Fig. 1), the first one numbered as (51), may contain such components. MS data and GC retention times of these fractions will be reported and discussed in a forthcoming paper[15].

TABLE III

COMPARISON OF RETENTION DATA OBTAINED IN THIS STUDY WITH CORRESPONDING LITERATURE DATA ACCORDING TO HOOD *et al.*[3]

Molecular weights as derived from *m/e* values of parent peaks of the 28 mass spectra obtained in this study are also listed. Fraction numbers and names of compounds printed in bold correspond to the tentative identifications by mass spectrometry shown in Table I.

Fraction No. (Fig. 1)	*Mol. wt.*	*Relative retention times*						*Compound*
		From this study				*From Hood et al.*[3]		
		40°	*84°*	*108°*	*155°*	*32°*	*90°*	
3	136	0.40				—		—
4	136	0.46				0.46		**α-Pinene**
4a	—	0.50				0.51		Camphene
—	—	—				0.62		2-Methyl-2-heptene-6-one
6	152	0.63				0.63		β-Pinene
6a	—	0.77				0.77		Myrcene
—	—	—				0.86		Car-3-ene
—	—	—				0.90		α-Terpinene
8	154	0.97				—		—
Limonene	(136)	1.01				1.00		Limonene
—	—	—				1.00		β-Phellandrene
—	—					1.14		*cis*-Ocimene
—	—					1.24		*trans*-Ocimene
8a	—	1.29				1.28		γ-Terpinene
—	—	—				1.63		Terpinolene
10	154	1.84	0.13			1.84		**Linalool**
11	154		0.14					**Fenchyl alcohol**
12	154		0.17					—
14	154		0.20					**Borneol**
15	154		0.21					—
16	154		0.23					**α-Terpineol**
18	150		0.62					**Piperitenone**
20	204		0.77	0.78				—
22	204		1.00	1.00			1.00	**β-Caryophyllene**
25	204			1.12			1.12	***trans*-α-Bergamotene**
26	204			1.19			1.19	**Humulene**
27	204			1.20			—	**Alloaromadendrene**
—	—			—			1.26	β-Farnesene
29	204			1.41				**α-Gurjunene**
30	204			1.49				—
31	204			1.68				—
33	204			1.93				—
36	222			2.29				—
38	222			2.61				—
39	222			2.82				—
41	220			3.05				—
43	220			3.52				—
44	222			3.82	1.00			—
48	220				1.70			—
50	238				2.20			—
51	—				9.3			—

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