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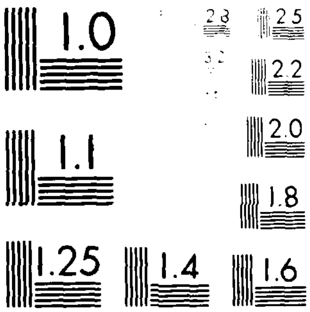

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DEPARTMENT OF DEFENCE

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MATERIALS RESEARCH LABORATORIES

MELBOURNE, VICTORIA

REPORT

MRL-R-836

AN EXAMINATION BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY OF THE HYDROCARBONS PRESENT IN THE ALGA BOTRYOCOCCUS BRAUNII

M.N. Galbraith, L.W. Hillen and L.V. Wake

Approved for Public Release

NOVEMBER, 1981

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The hydrocarbons present in a number of 'blooms' of *Botryococcus braunii* from different Australian sites have been examined by gas chromatography-mass spectrometry. This organism was found to contain many novel branched-chain hydrocarbons of the type C_n-H_{2n-10} where n equals 30, 34, 35, 36 or 37. These compounds were present in both red and green 'blooms' of the alga.

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The hydrocarbons present in a number of 'blooms' of *Botryococcus braunii* from different Australian sites have been examined by gas chromatography-mass spectrometry. This organism was found to contain many novel branched-chain hydrocarbons of the type C_n-H_{2n-10} where n equals 30, 34, 35, 36 or 37. These compounds were present in both red and green 'blooms' of the alga.

CONTENTS

AN EXAMINATION BY GAS CHROMATOGRAPHY-MASS SPECTROMETRYOF THE HYDROCARBONS PRESENT IN THE ALGA BOTRYOCOCCUS BRAUNII

1. INTRODUCTION

It has generally been observed that hydrocarbon biosynthesis by microscopic algae produces small amounts of aliphatic hydrocarbons with a relative maximum in chain length of about C_{17} [1]. The colonial alga *Botryococcus braunii* Kützing differs, however, in that a green stage of the organism has been observed to contain longer straight chain hydrocarbons in the range C_{27}-C_{31} [2] in concentrations up to 17% of the total dry weight [3]. In addition, two unsaturated branched-chain hydrocarbons have been observed in high concentration in the red resting stage of the alga. These two branched-chain hydrocarbons, which bear no apparent structural relationship to the straight chain compounds, have been reported to comprise up to 75% of the total dry weight of the organism [4].

'Blooms' of *B. braunii* were sampled and the hydrocarbon compositions analysed by gas chromatography-mass spectrometry (GC-MS). Many branched-chain hydrocarbons were observed, some of which have not been recorded previously. The purpose of this paper was to compile a mass spectral index of the isolated hydrocarbons as a reference for further biochemical studies. The hydrocarbon content of the 'blooms', which was sufficient to cause the individual colonies to float at the surface of the various water bodies, was also measured.

2. MATERIALS AND METHODS

2.1 *Hydrocarbons*

The harvested algal suspensions were frozen and subsequently transferred to a 'Dynavac' two litre freeze drying unit in preparation for extraction of the hydrocarbons. The freeze-dried algal cells were subjected to exhaustive extraction using a soxhlet apparatus and acetone as solvent [2]. The extraction was continued until the fresh extract solution was almost colorless. The solvent was removed under vacuum leaving a dark green oil from which the hydrocarbon fraction was separated by liquid chromatography with alumina (activity 1) as the stationary phase. Elution with a light petroleum fraction initially gave a colorless oil, however later fractions of the oil were yellow to orange in colour. This process was continued until the low polarity material had been eluted leaving a green residue on the column. The solvent was then removed in a rotary evaporator.

The algal hydrocarbons were analysed by gas chromatography and gas chromatography-mass spectrometry (GC-MS). Fifty metre SCOT columns were employed with an i.d. of 0.05 mm; the columns, coated with SE-30 were capable of 40,000-50,000 theoretical plates. The column temperature was programmed from 200°C to 280°C at 4°C per minute with a helium flow rate of 4 mL per minute. A Finnigan 3300-E GC-MS equipped with chemical ionisation using methane gas was used with a 6100 data collection system. Retention index data was obtained by co-injection with standard hydrocarbon mixtures. Due to interference from the C_{28} standard hydrocarbon, retention indices in the range 2790-2810 were estimated from the GC-MS record.

3. RESULTS AND DISCUSSION

3.1 Hydrocarbons from B. braunii

The hydrocarbon content of colonies of *B. braunii* sampled in this programme ranged from 27% to 40% on a dry weight basis. The liquid hydrocarbon content was generally lower in the green form and higher in the red form of the alga as has been observed by other workers. While the average concentration of 30% for the green form was lower than that observed in the red form, it is nearly double that previously reported [3] for this stage of the alga. The higher hydrocarbon content (40%) of the red colonies is approximately half that recorded [4] for a red 'bloom' which occurred in Oakmere, Cheshire during 1965.

Red 'blooms' of the alga were observed to contain only branched-chain hydrocarbons with the exception of the Devilbend 'bloom', see Table 2, which also contained traces of linear compounds. It is believed that these were possibly due to the presence of a small number of green colonies present in a much larger concentration of dark reddish colonies. Green 'blooms', on the other hand, were observed to contain straight or branched-chain compounds although it was earlier reported [2] that only straight chain compounds were present in this stage of the alga.

A number (ca. 13) of previously unreported branched-chain hydrocarbons were observed in two of the 'blooms' examined, namely a green 'bloom' present in the Darwin River Reservoir and a yellow to red 'bloom' present in the Devilbend Reservoir. All of these hydrocarbons are of the general formula C_nH_{2n-10} where n = 30, 34, 35, 36 and 37. Some of these compounds were present as major components while others were present in traces. Several of the hydrocarbons were common to both 'blooms'. A number of unidentified compounds were also present in concentrations below that permitting determination of their molecular weights from the mass spectra. The hydrocarbon types and percentage compositions present in each 'bloom' are described below and listed in Tables 1 and 2.

The hydrocarbons found are based on the collective contribution of a large number of cells. This average composition therefore does not necessarily hold for individual cells. The presence of traces of linear hydrocarbons in the Devilbend 'bloom' may therefore be due to a contribution from a small number of green colonies, as suggested above, rather than being characteristic of the large mass of dark red cells. Similarly the branched-chain hydrocarbons observed in the Sorrento 'bloom' may be present more as a consequence of the smaller number of red cells than of the larger number of green colonies. There is preliminary evidence to suggest that this is the case, viz. colonies selectively separated from the 1976 Sorrento sample collected in 1976 had a markedly different composition to the bulk of the material (see Table 1).

Figures 1 to 22 provide reference mass spectra of the hydrocarbons observed in *B. braunii* up to the present time. Eight of these compounds have been reported previously and 13 are new. The mass spectra have been denoted by retention indices as the structures of most compounds have yet to be determined. The relative intensities of the six most intense peaks in each spectrum are listed in Table 3.

3.2 Chemical Ionization

In GC-MS operation the chemical ionization technique allows molecular ions to be observed more readily. This is an advantage when dealing with highly branched compounds such as those under study where the molecular ions are quite weak using electron impact ionization. In many instances when using methane for chemical ionization, a "pseudo molecular" ion at a mass corresponding to the molecular mass + 1 is obtained as well as further peaks 15 and 29 mass units higher than the molecular mass. These are summation peaks of the molecular mass plus the mass of CH_3^+ and $C_2H_5^+$ fragments. When observed, these peaks provide additional evidence for the assignment of molecular mass (refer figures 8, 12, 16, 17, 21 and 22). Thus the molecular mass of one C_{37}, two C_{36} and two C_{34} compounds are established beyond question.

3.3 Computer Matching

The mass spectrum is not only a function of the compound itself but of the mass spectrometer design, the mode of operation and in the case of chemical ionization spectra, the type and pressure of the reactant gas. A comparative match technique [5] was employed to provide a figure of merit for spectral matching. The combination of gas chromatographic retention index and the mass spectrum for each hydrocarbon provides the easiest method of identification.

The matching technique, based on a 'Divergence Analysis', compares the mass numbers and relative intensities of the largest peaks of the mass spectrum; e.g. 'the Big 4 or 6' method compares the four or six largest peaks [6].

While qualitatively useful for confirming the similarity of the hydrocarbons from different sources, the 'Divergence Analysis" technique yields better results if an optimum 'Big N' programme is used. The value of N is selected such that there is a marked intensity gap between the N and N+1 peaks, e.g. when comparing the mass spectra of botryococcene samples, a 'Big 4' analysis gives better results than a 'Big 6' analysis since the 5th to the 10th most intense peaks of botryococcene are of similar intensity and markedly less intense than the 4th peak (Fig. 8, Table 4). The same applies to the mass spectrum of isobotryococcene (Fig. 7, Table 5). On the other hand in the case of squalene the 'Big 6' analysis is appropriate since the 4th, 5th and 6th peaks are all of similar intensity. (Figs. 13, 22; Table 6). The computer matching technique proved very useful for the identification of squalene as a component of the hydrocarbons extracted from the Darwin and Devilbend 'blooms' of *B. braunii* (as well as from a Coorongite distillate).

A data bank of the mass spectra of all the hydrocarbons extracted from *B. braunii* was prepared and each compound was set as an unknown against the data bank thus allowing the hydrocarbons to be tested for spectral similarity. When the hydrocarbons are matched in this way they fall into three broad groups:-

(1) Linear hydrocarbons (Table 7)

(2) Botryococcene branched types (Table 8)

(3) non-Botryococcene branched types (Tables 6,9,10)

As well as the three broad groups the mass spectrum of squalene was observed together with another spectrum which could not be classified by the computer matching technique spectra.

It is inferred that the above grouping (see also Table 11) relates to structural similarities among members of each group and structural differences between groups.

It is interesting to note that 'Big 4 and Big 6' cannot distinguish between botryococcene and isobotryococcene which are known [4] to have the same carbon skeletons. The structural differences between these compounds are such as to have only a small influence on the mass spectrum and thus the retention indices are also needed to differentiate positively between these two hydrocarbons.

Table 7 lists mass spectra that exhibit a high odd/even ratio in the chain lengths of the various fragments produced. In this comparison different compounds exhibit marked similarities or differences depending on whether a 'Big 4 or Big 6' analysis is used. A number of compounds within this group show extremely close similarities when compared to one other by 'Divergence Analysis', e.g. the Devilbend samples ($C_{34}H_{58}$, R.I. 2790, Fig. 14 and $C_{36}H_{62}$, R.I. 2908, Fig. 18) although differing substantially from the reference compound for this group, i.e. the Devilbend hydrocarbon ($C_{34}H_{58}$, R.I. 2752, Fig. 11).

The mass spectrum of one of the hydrocarbon compounds could not be classified on the basis of computer matching. Its mass spectrum (Fig. 9) resembles branched chain hydrocarbons (e.g. Fig. 21) in appearance although it also correlates with straight chain compounds (e.g. Fig. 1) by Divergence Analysis. The major ion fragment in this spectrum, at mass 111, belongs to the series C_nH_{2n-1} characteristic of linear hydrocarbons, whereas in the botryococcene series the major ion fragment is a member of the C_nH_{2n-3} series (e.g. mass 109).

3.4 Comments of Hydrocarbon Results

The hydrocarbon pattern produced by *B. braunii* is more complex than earlier researchers [3] suspected and raises questions as to the cause of this variation. The presence of squalene, also reported in *B. braunii* by Gelpi *et al* [7], suggests the question of the involvement of this compound in a biosynthetic pathway for the hydrocarbons akin to that usually considered for steroid synthesis. It may be relevant that C4-C6 acids are present in the evaporate from freeze-drying of the cells which give it an opalescent appearance and a prominent odour distinctive of these C4-C6 compounds.

The presence of iso-(2-methyl) and anteiso-(3-methyl) hydrocarbons in geological samples is generally explained by their occurrence in plant waxes [9] and by the presence of branched chain acids in bacteria and marine algae [10]. The presence of these hydrocarbons in *B. braunii* raises the question of this alga's possible contribution to earlier reports on geological accumulations of branched-chain hydrocarbons particularly in the light of the extensive fossil record of *B. braunii* [11] and the appearance of botryococcane in oil samples [12].

3.5 Reservoirs Examined

3.5.1 Tarago Reservoir

A small 'bloom' of *B. braunii* occurred in the Tarago Reservoir over a six month period between March and September, 1975. Algal harvesting was carried out on 15/5/75 and 12/8/75. The liquid hydrocarbon content of the grass-green algal colonies collected in Tarago Reservoir is shown in Table 1 together with the levels observed in the Sorrento samples. GC-MS analysis of the hydrocarbon components from the two Tarago collections showed a composition within 1% of each other. There were three major and three minor peaks of straight chain hydrocarbons of the general formula C_nH_{2n-x} where $n = 25, 27, 29$ or 31 and $x = 2$ or 4. This composition resembles that observed [2] for the green stage of *B. braunii*.

3.5.2 Sorrento Water Tower

'Blooms' of *B. braunii* occurred in the two storage towers at Sorrento during February 1976 and March 1978 and were sampled over the period 4-6/2/76 and on 22/2/78. Gas chromatographic analysis of the liquid hydrocarbons extracted from the colonies indicated a composition in which both straight chain and branched-chain hydrocarbons were present (Table 1). Liquid chromatography on silver impregnated alumina columns retarded the branched-chain material, permitting separation from the straight chain hydrocarbons. On standing, a small fraction of predominantly red cells separated from the bulk of the sample. The hydrocarbon composition of this sample differed from the bulk of the material in that a greater proportion of branched-chain hydrocarbons was present. This difference shows that there is a heterogeneity in the hydrocarbon composition between algal colonies in the 1976 'bloom'. As the red cells were not entirely free of the green form, it was not possible to establish whether the red cells contained linear hydrocarbons nor whether the green cells contained branched-chain compounds. GC-MS indicated that branched-chain material was absent in samples taken from the 'bloom' occurring in 1978.

3.5.3 Darwin River Reservoir

A 'bloom' of *B. braunii* appeared in the Darwin River Reservoir over the period 1976-9. Sampling was carried out over 4-7/11/76 and again on 18-28/10/77. An additional sample was received [13] in August 1978. The content of liquid hydrocarbons extracted from the organism is shown in Table 2 together with the oil assays from the 'blooms' in Devilbend Reservoir, McCay's Reservoir and Green Lake. Minor changes in composition were seen between the samples harvested at Darwin. GC-MS showed that all of the major hydrocarbon compounds present in the oil from the 'bloom', a green form of the plant, were unexpectedly branched-chain compounds not previously observed in this green form of the alga. As mentioned above, earlier studies had reported that straight chain unsaturated compounds were present in this form of the alga [2]. The mass spectra and GC retention indices of the first two chromatographic peaks correspond with samples of the $C_{34}H_{58}$ hydrocarbons, botryococcene and isobotryococcene [14]. A third $C_{34}H_{58}$ isomer was also present, all three isomers being present as minor components. A trace of the $C_{30}H_{50}$ compound, squalene, was eluted after the last C_{34} peak. Identification was confirmed by comparison with a reference sample of squalene ran under the same conditions of operation of the GC-MS. The major component of the oil was an unidentified compound with a molecular mass of 494 corresponding to $C_{36}H_{62}$. A second C_{36} hydrocarbon was present in a complex chromatograhic peak together with a compound of molecular mass 508 corresponding to $C_{37}H_{64}$. A second C_{37} hydrocarbon was present in a peak which may have a further C_{36} compound therein. All of the hydrocarbons from Darwin analysed by GC-MS were homologues of the general series C_nH_{2n-10} where n equals 30, 34, 36 and 37.

3.5.4 McCay's Reservoir

A red 'bloom' of *B. braunii* was reported in McCay's Reservoir in May, 1977. When the dam was visited on 9/5/77 the 'bloom' had largely disappeared and lysed cell debris was present in a surface scum. Extraction and chromatography of material taken from the reservoir and freeze-dried produced a hydrocarbon oil which constituted 32% of the dry weight (Table 2). However it is felt that this figure would have been considerably higher had the material been free of extraneous debris. Chromatography of the oil showed three peaks present, the first corresponding to either botryococcene or one of the C_{34} hydrocarbons observed in the Devilbend sample. The second peak is a complex of two compounds both of which are thought to be C_{36} branched-chain hydrocarbons of the botryococcene type. Both of these correspond to peaks present in the Devilbend sample. The third peak corresponds to a C_{37} hydrocarbon also present in the Devilbend sample.

3.5.5 Green Lake

In December, 1976 an orange coloration was reported which covered about 25% of the lake surface. This orange material was sampled and found to have a water content of 38% w/w. Microanalysis for solvent extractible oils (extracted by carbon disulphide and analysed by gas chromatography using the linear hydrocarbons as standards) gave 27 ± 3% hydrocarbons on a dry weight basis (Table 2). Chromatography of the oil showed that one major and two minor peaks were present. GC-MS analysis indicated the first peak to be isobotryococcene and the major constituent to be botryococcene (Table 2). These two peaks correspond with samples supplied by Dr. J. Maxwell (Table 4). The third peak was an unsaturated compound but from the mass spectrum is not thought to be a hydrocarbon.

3.5.6 Devilbend Reservoir

A reddish 'bloom' of the alga *B. braunii* was observed and sampled from the Devilbend Reservoir on 22/2/78. The liquid hydrocarbon content of 40% of the total dry weight of *B. braunii* colonies from Devilbend Reservoir was the highest observed in the present series. Chromatography of the oil from this red stage showed that there were 11 hydrocarbon compounds present (Figure 2b). GC-MS revealed a marked similarity in composition to the hydrocarbons obtained from the green colonies of the Darwin 'bloom' (Table 2). Isobotryococcene and another C_{34} isomer were common to both 'blooms', as were squalene and C_{36} hydrocarbons present as the major component in the Darwin oil. One $C_{37}H_{64}$, two $C_{36}H_{62}$ and two $C_{34}H_{58}$ hydrocarbons that were present in this 'bloom' were absent in the Darwin 'bloom'. The absence of botryococcene from this 'bloom' was most intriguing.

4. CONCLUSIONS

Under Australian conditions 'blooms' of *B. braunii* may contain a hydrocarbon oil content between 27% and 40% of the plant's dry mass. The highest oil content was observed in a red 'bloom' and the lowest in a green 'bloom' of the organism.

A number of novel branched-chain hydrocarbons of the general formula C_nH_{2n-10} where n equals 30, 34, 35, 36 and 37 have been found in both red and green 'blooms' of the alga. Analysis by GC-MS indicates that these compounds are closely related to botryococcene.

Branched-chain compounds were present in both red and green 'blooms' whereas straight chain hydrocarbons were present only in the green stage of the organism. Whether linear and branched-chain hydrocarbons occur simultaneously in the plant is inconclusive as 'blooms' which revealed both types of hydrocarbons were known to contain both stages of the alga.

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TABLE 1

HYDROCARBONS FROM ALGAL BLOOMS OF B. BRAUNII

RETENTION INDEX	COMPOUND	FIGURE NO.	MOLECULAR MASS	PERCENTAGE COMPOSITION			
				TARAGO	SORRENTO GREEN 76	SORRENTO RED 76	SORRENTO 78 SAMPLE
2467	$C_{25}H_{48}$	1	348	2	2	1	7
2666	$C_{27}H_{50}$	-	374	3	-	-	-
2673	$C_{27}H_{52}$	2	376	40	18	-	56
2858	$C_{29}H_{54}$	3	402	13	37	27	23.5
2874	$C_{29}H_{56}$	4	404	37	"	"	6.5
3046	$C_{31}H_{58}$	5	430	-	16	8	4
3071	$C_{31}H_{60}$	6	432	5	"	"	3
3237^{+}	-	-	-	-	27	64	-
TOTALS				100	100	100	100
PERCENTAGE OIL CONTENT				30 ± 3	27	NE	30

NE = Not Examined

$^{+}$ not positively identified by MS

Inverted commas indicate peak not resolved from immediately preceding figure.

TABLE 2

BRANCHED-CHAIN HYDROCARBONS FROM ALGAL BLOOMS OF B. BRAUNII

RETENTION INDEX	COMPOUND FORMULA	FIGURE NO.	MOLECULAR MASS	PERCENTAGE COMPOSITION					
				DEVILBEND	DARWIN NOV 76	DARWIN AUG 77	DARWIN OCT 77	GREEN LAKE	McCAY'S RESERVOIR
2662	$C_{27}H_{52}$	2	276	0.4	-	-	-	-	-
2688-92	-		(?)	0.2	0.7	0.2	trace	-	-
2707-9	$C_{34}H_{58}$	7	466	2.8	4.4	4.3	3.3	10.1	-
2728-31	$C_{34}H_{58}$	8	466	-	9.7	8.9	8.0	85.7	-
2727	$C_{34}H_{58}$	9	466	3.7	-	-	-	-	42.
2732	$C_{34}H_{58}$	10	466+(452?)	3.7	-	-	-	-	-
2741	nonhydrocarbon		452	-	-	-	-	4.2	-
2752	$C_{34}H_{58}$	11	466	2.5	-	-	-	-	-
2758-62	$C_{34}H_{58}$	12	466	-	11.5	10.2	14.6	-	-
2784-5	$C_{30}H_{50}$	13	410	0.8	1.6	-	0.4	-	-
2790^{+}	$C_{34}H_{58}$	14	466	trace	-	2.7	-	-	-
2798(+)	$C_{35}H_{60}$(?)	15	480	-	-	trace	-	-	-
2816-8	(?)	(?)		-	2.7	1.5	0.6	-	-
2825	(?)	(?)		0.4	-	-	-	-	-
2845-49	(?)	(?)		-	0.9	0.5	-	-	-
2853	$C_{29}H_{56}$	4	404	0.4	-	-	-	-	-
2869-70	(?)	(?)		-	1.2	0.5	0.8	-	-

TABLE 2
(Continued)

RETENTION INDEX	COMPOUND FORMULA	FIGURE NO.	MOLECULAR MASS	PERCENTAGE COMPOSITION					
				DEVILBEND	DARWIN NOV 76	DARWIN AUG 77	DARWIN OCT 77	GREEN LAKE	McCAY'S RESERVOIR
2878	$C_{36}H_{62}$	16	494	32.2	-	-	-	-	42.0
2913-20	$C_{36}H_{62}$	17	494	36.5	36.5	31.9	53.8	-	-
2908	$C_{36}H_{62}$	18	494	(?)	-	-	-	-	-
2931-39	$C_{36}H_{62}$	19	494	6.9	4.8	4.1	0.7	-	-
2957-64	$C_{37}H_{64}$	20	508	-	17.4	26.5	16.9	-	-
2957-64	$C_{37}H_{64}$		508	0.9	-	-	-	-	-
2974-6	$C_{37}H_{64}$	21	508	7.8	-	0.4	0.9	-	16.0
2983	-		-	-	4.1	2.8	-	-	-
3000-2	-		(?)	0.8	-	2.0	-	-	-
3019-20	-		(?)	-	1.6	0.8	-	-	-
3069-71	-		-	-	1.6	-	-	-	-
Others	-		-	-	1.3	2.7	-	-	-
TOTALS				100	100	100	100	100	100
PERCENTAGE OIL CONTENT				40	33	NE++	29	27	32

\+ Retention index estimated from GC-MS record

NE++ Not examined

TABLE 3

MASS SPECTRAL DATA FOR THE HYDROCARBONS OF BOTRYOCOCCUS - RELATIVE INTENSITIES OF 6 MAJOR MASS FRAGMENTS

Figure Number	Major Ion	Mass Number/Intensity of 6 Major Peaks	Sample* Origin	Spectrum Code	Carbon Atoms	Retention Index
1	97	71/21,83/52,97/99,111/57,125/38,139/25	SOR 78	155-151	25	2467
2	97	71/25,83/51,97/99,111/63,125/38,139/22	SOR 78	208-202	27	2473
3	97	77/70,83/59,97/99,109/59,111/42,125/31	SOR 78	271-265	29	2858
4	97	71/25,83/48,97/99,111/68,125/41,139/24	SOR 78	275-272	29	2874
5	97	83/60,97/99,109/38,111/56,125/38,137/35	SOR 78	358-353	31	3046
6	97	83/52,96/23,97/99,111/61,125/49,139/29	SOR 78	369-363	31	3071
7	109	69/45,95/92,109/99,123/83,125/72,137/53	DARK H77	1-6	34	2708
8	109	95/83,109/99,111/42,123/75,125/64,137/46	DARK H77	11-6	34	2730
9	111	95/51,97/42,109/62,111/99,123/45,125/39	DBN 78	214-211	34	2727
10	109	95/82,109/99,123/95,137/49,151/62,177/55	DBN 78	219-216	34	2732
11	123	95/80,109/91,123/99,125/69,151/99,177/83	DBN 78	226-223	34	2752
12	123	95/76,109/92,123/99,125/68,151/90,177/57	DARK H77	31-26	34	2762
13	69	69/99,81/99,95/49,109/87,123/61,137/99	DBN 78	238-236	30	2785
14	151	95/25,109/32,123/78,139/36,151/99,177/49	DBN 78	242-239	34	2890
15	109	83/51,95/47,109/99,111/47,123/68,125/63	DARK H77	62-56	35	2798
16	109	95/92,97/65,109/99,123/96,125/77,137/76	DBN 78	265-259	36	2879
17	109	95/84,97/66,109/99,123/85,125/84,137/60	DAR 77	276-271	36	2913
18	151	111/31,123/63,137/32,139/43,151/99,177/79	DBN 78	280-279	36	2913-20
19	123	97/60,109/75,123/99,137/60,151/99,177/75	DARK H77	146-140	36	2939
20	109	83/65,95/89,109/99,111/86,123/78,125/83	DAR 77	302-298	37	2957
21	95	95/99,109/97,111/66,123/89,125/85,137/78	DBN 78	307-304	37	2976
22	137	69/80,81/96,95/39,109/43,123/38,137/99	Squalene		30	2793

ORIGIN CODE

SOR 78	Sorrento Water Tower, 1978
DARK H77	Darwin River Reservoir, Aug. 1977
DAR 77	Darwin River Reservoir, Oct. 1977
DBN 78	Devilbend Reservoir, 1978

TABLE 4

MATCHING OF SPECTRA OF BOTRYOCOCCENE FROM VARIOUS SOURCES

'BLOOM'	RETENTION INDEX	DIVERGENCE (4)*	DIVERGENCE (6)*
Maxwell+	2731	0.000	0.000
Darwin Oct '77	2728	< 0.001	0.187
Green Lake	2731	< 0.001	0.093
++Darwin Aug '77+	2730	< 0.001	0.000
Darwin Nov '76	2730	0.001	0.194

\+ Mass spectra obtained on the same day

++ Compound used for identification - see Figure 8

* Divergence determined by 'Big 4' or 'Big 6' technique

TABLE 5

MATCHING OF MASS SPECTRA OF ISOBOTRYOCOCCENE FROM VARIOUS SOURCES

'BLOOM'	RETENTION INDEX	DIVERGENCE (4)*	DIVERGENCE (6)*
Maxwell	2710	0.000	0.000
Darwin Oct '77	2707	0.001	0.093
Green Lake	2708	0.000	0.097
++Darwin Aug '77+	2708	0.000	0.092
Darwin Nov '76	2708	0.001	0.007
Devilbend	2708	0.000	0.113

\+ Mass spectra obtained on same day

++ Compound used for identification - see Figure 7

* Divergence determined by 'Big 4' or 'Big 6' technique

TABLE 6

MATCHING OF THE MASS SPECTRA OF SQUALENE

SOURCE	COMPOUND	FIGURE NO.	RETENTION INDEX	DIVERGENCE (4)	DIVERGENCE (6)
Devilbend	Squalene	22	2784	0.000	0.000
Squalene	'Lab'	13	2785	0.007	0.007

TABLE 7

MATCHING OF HYDROCARBONS WITH BOTRYOCOCCENE TYPE MASS SPECTRA

SOURCE	COMPOUND	FIGURE NO.	RETENTION INDEX	DIVERGENCE (4)	DIVERGENCE (6)
Darwin '77	$C_{34}H_{58}$	8	2730	0.000	0.000
Darwin '77	$C_{34}H_{58}$	7	2708	0.000	0.089
Devilbend	$C_{36}H_{62}$	16	2879	0.001	0.107
Darwin '77	$C_{36}H_{62}$	17	2913	0.001	0.111
Devilbend	$C_{37}H_{64}$	21	2976	0.001	0.004
Devilbend	$C_{34}H_{58}$	10	2732	0.180	0.245
Darwin '77	$C_{35?}H_{60}$	15	2798	0.213	0.119
Darwin	$C_{37}H_{64}$	20	2957	0.235	0.115
Darwin '77	$C_{34}H_{58}$	12	2762	0.261	0.247

TABLE 8

MATCHING OF MASS SPECTRA OF STRAIGHT CHAIN HYDROCARBONS

SOURCE	COMPOUND	FIGURE NO.	RETENTION INDEX	DIVERGENCE (4)	DIVERGENCE (6)
Sorrento	$C_{29}H_{56}$	4	2874	0.000	0.000
Sorrento	$C_{27}H_{52}$	2	2673	0.000	0.000
Sorrento	$C_{25}H_{48}$	1	2467	0.001	0.001
Sorrento	$C_{31}H_{60}$	6	3071	0.001	0.061
Sorrento	$C_{31}H_{58}$	5	3046	0.136	0.162
Sorrento	$C_{29}H_{54}$	3	2858	0.421	0.251
Devilbend	$C_{34}H_{58}$	9	2727	0.661	0.389

TABLE 9

MATCHING OF SPECTRA WITH HIGH ODD/EVEN CHAIN LENGTH FRAGMENTS[+]

SOURCE	COMPOUND	FIGURE NO.	RETENTION INDEX	DIVERGENCE (4)	DIVERGENCE (6)
Devilbend	$C_{34}H_{58}$	11	2752	0.000	0.000
Darwin '77	$C_{36}H_{62}$	19	2939	0.001	0.258
Devilbend	$C_{34}H_{58}$	14	2790	0.182	0.130
Devilbend	$C_{36}H_{62}$	18	2908	0.191	0.294
Darwin '77	$C_{34}H_{58}$	12	2762	0.200	0.002
Devilbend	$C_{34}H_{58}$	10	2732	0.233	0.117
Darwin '77	$C_{35?}H_{60}$	15	2798	0.438	0.302
Darwin '77	$C_{34}H_{58}$	8	2730	0.469	0.274
Devilbend	$C_{36}H_{62}$	16	2879	0.472	(?)
Darwin '77	$C_{34}H_{58}$	7	2708	0.478	0.277

[+] Spectra have an alternating appearance in fragment intensities

TABLE 10

MASS SPECTRA OF UNGROUPED COMPOUND c.f. OTHER SPECTRA

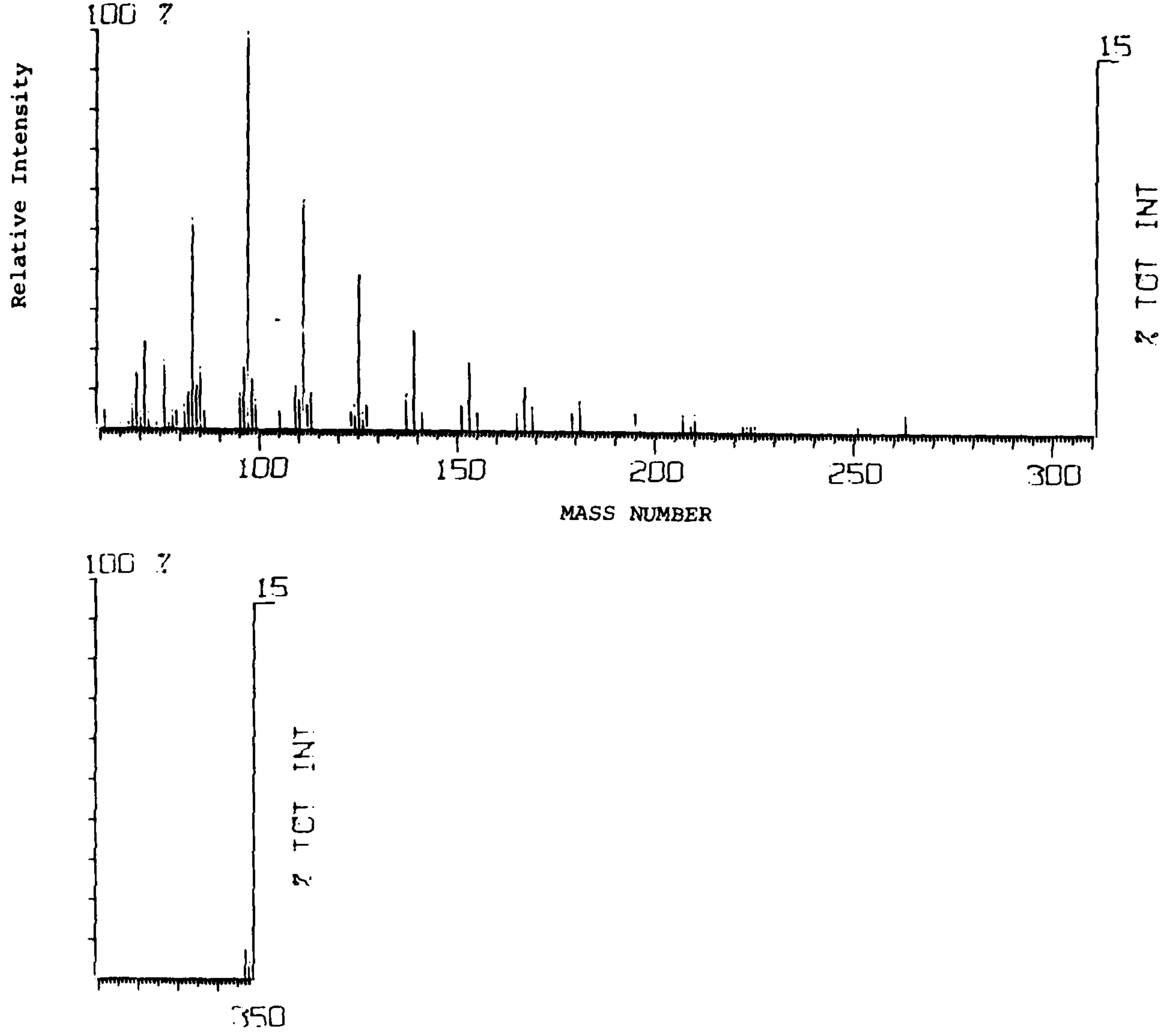
SOURCE	COMPOUND	FIGURE NO.	RETENTION INDEX	DIVERGENCE (4)	DIVERGENCE (6)
Devilbend	$C_{34}H_{58}$	9	2727	0.000	0.000
Darwin '77	$C_{37}H_{64}$	20	2957	0.202	0.126
Devilbend	$C_{34}H_{58}$	10	2732	0.307	0.455
Darwin '77	$C_{34}H_{58}$	8	2730	0.313	(?)
Darwin '77	$C_{34}H_{58}$	7	2708	0.319	0.330
Devilbend	$C_{36}H_{62}$	16	2879	0.322	0.246
Devilbend	$C_{37}H_{64}$	21	2976	0.332	0.151
Darwin '77	$C_{36}H_{62}$	17	2913	0.336	0.233
Darwin '77	$C_{35?}H_{60}$	15	2798	0.503	0.146
Sorrento	$C_{25}H_{48}$	1	2467	0.687	0.405
Sorrento	$C_{29}H_{54}$	3	2858	(?)	0.398
Sorrento	$C_{29}H_{56}$	4	2874	0.661	0.389

TABLE 11

CLASSIFICATION OF MASS SPECTRAL TYPES

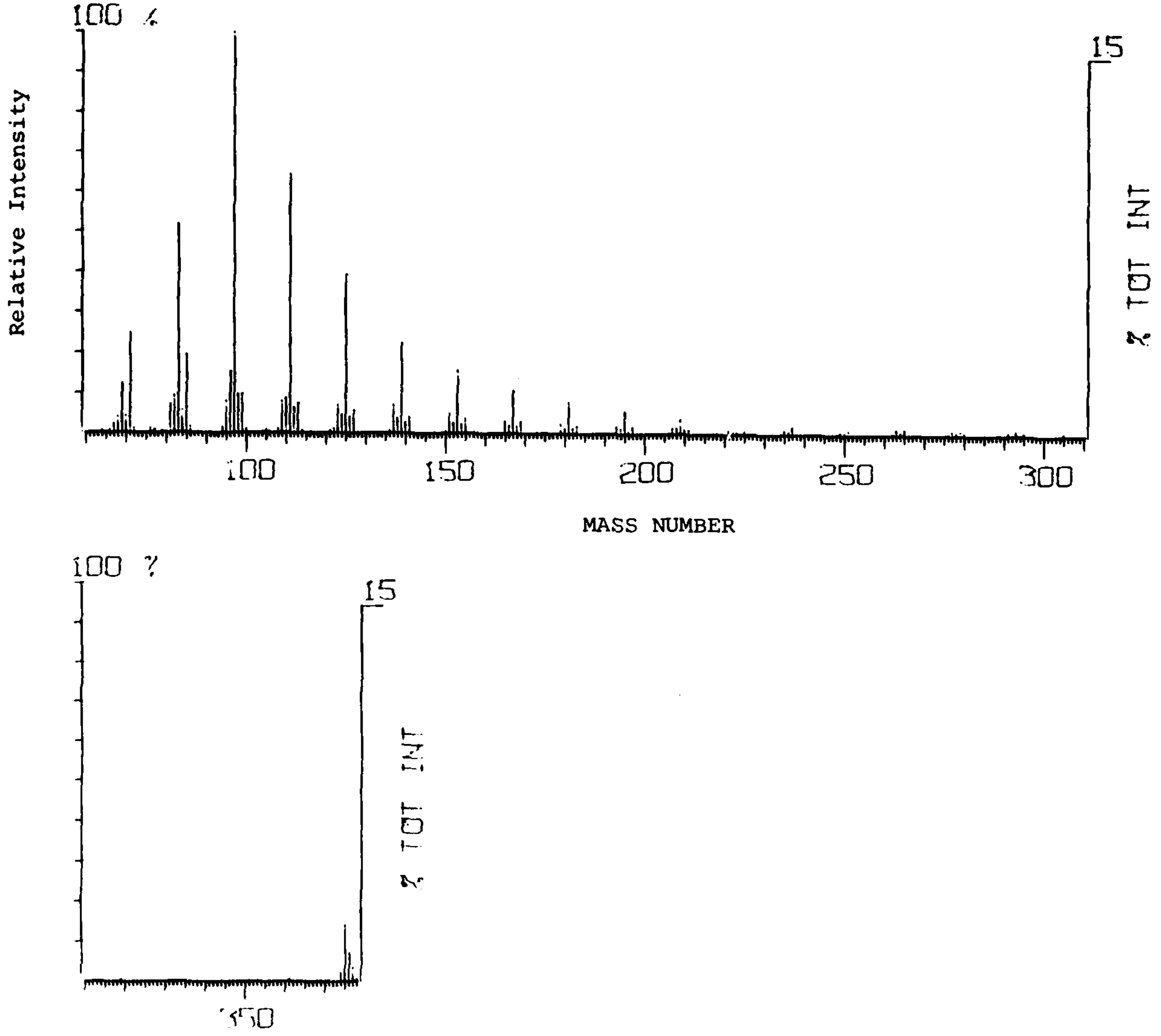
MASS SPECTRAL PATTERN	FIG. NUM.	STRUCTURAL TYPE
Linear Hydrocarbons	1,2,3,4,5,6	Linear carbon skeleton.
Squalene	13,22	Branched carbon skeleton; double bonds located in main chain
Botryococcene type	7,8,16,17,20,21	Branched chain skeleton. Most double bonds located in side chains
non-Botryococcene type	11,14,18,19(10,12,15)	Branched carbon skeleton characterised by prominent peaks at mass nos. 123, 151 and 171
Ungrouped type	9	Characteristics of both straight and branched chain hydrocarbons

FIGURE 1



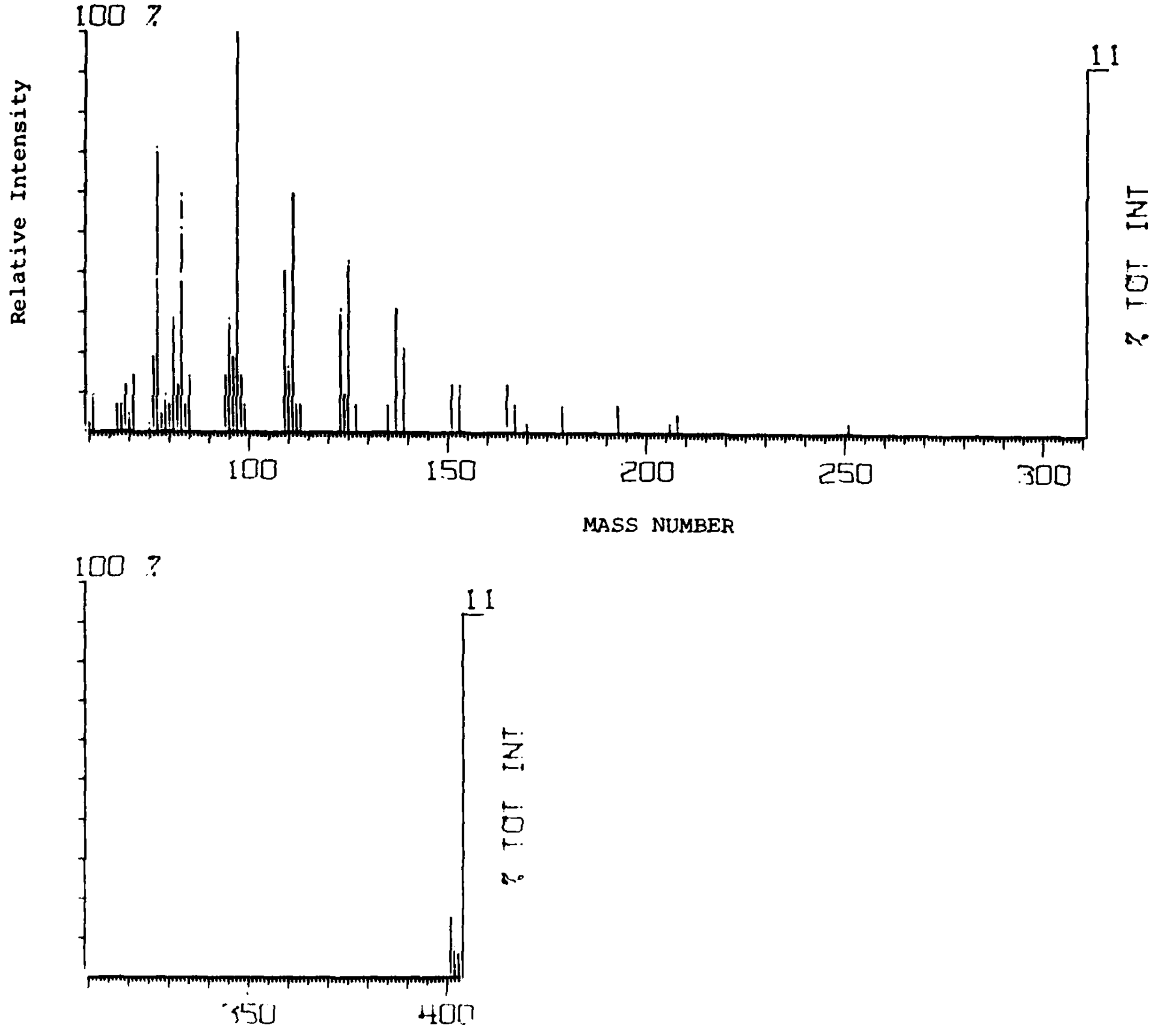
$C_{25}H_{48}$ R.I. 2467

FIGURE 2



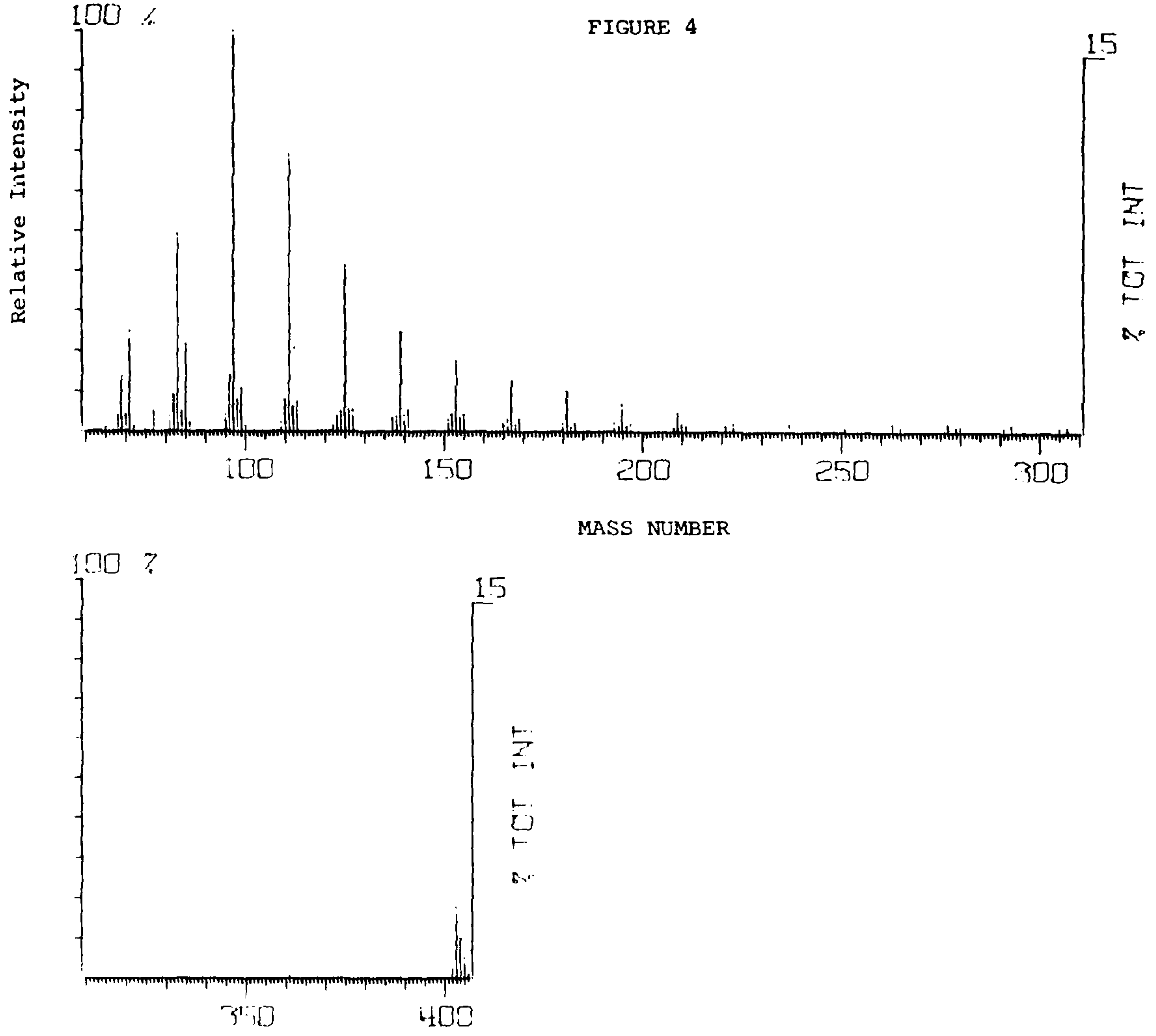
$C_{27}H_{52}$ R.I. 2673

FIGURE 3



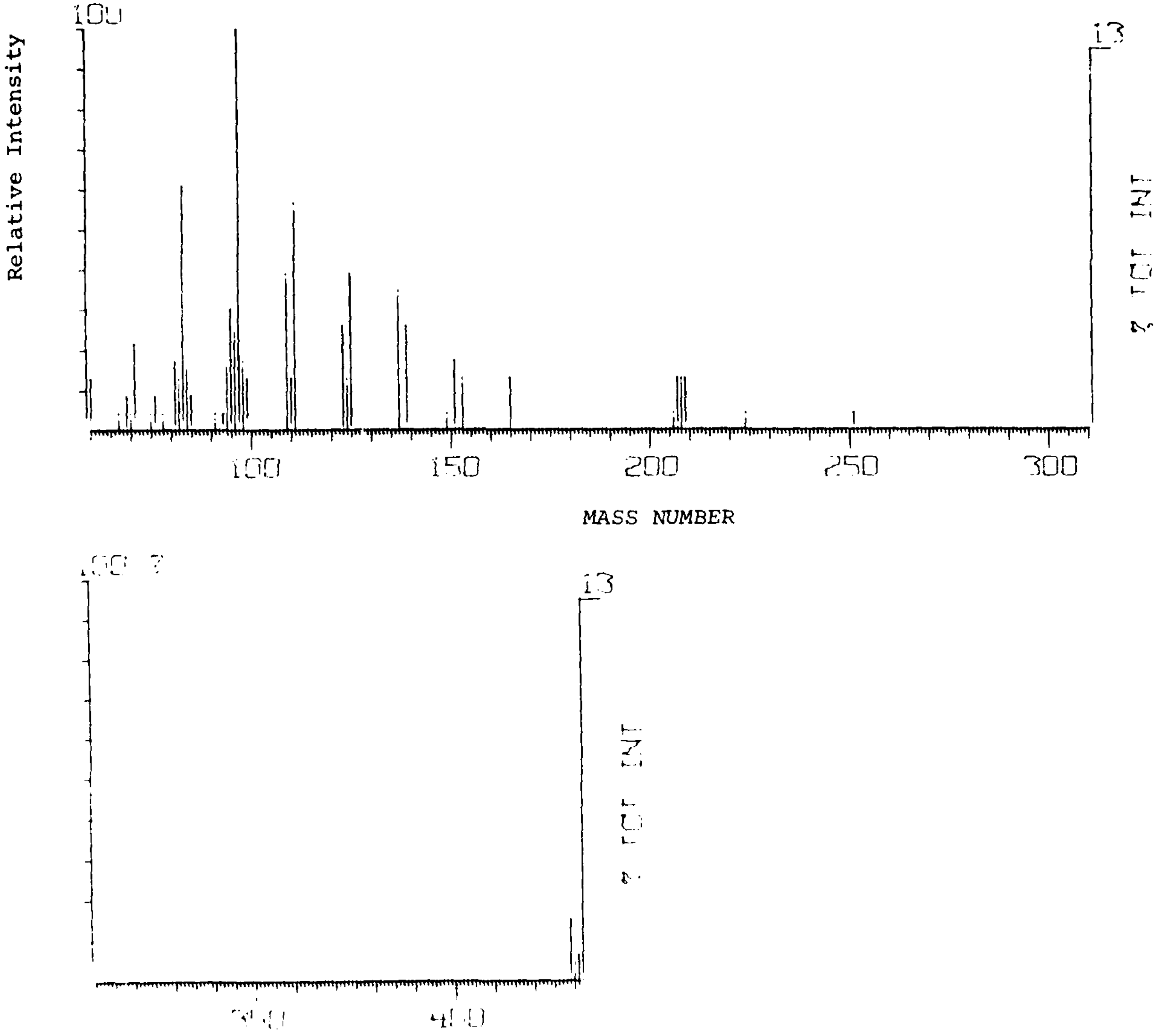
$C_{29}H_{54}$ R.I. 2858

FIGURE 4



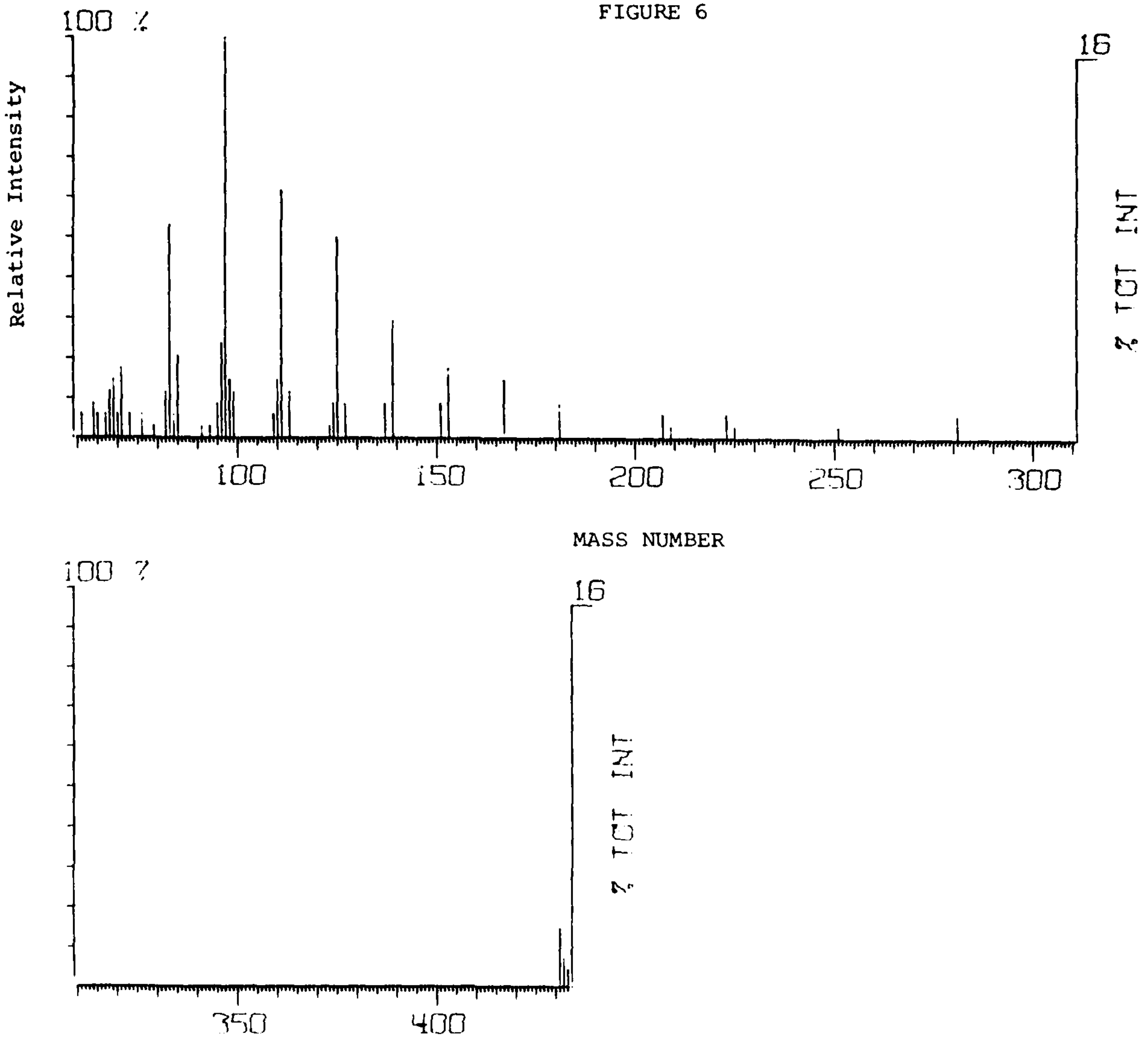
$C_{29}H_{56}$ R.I. 2874

FIGURE 5



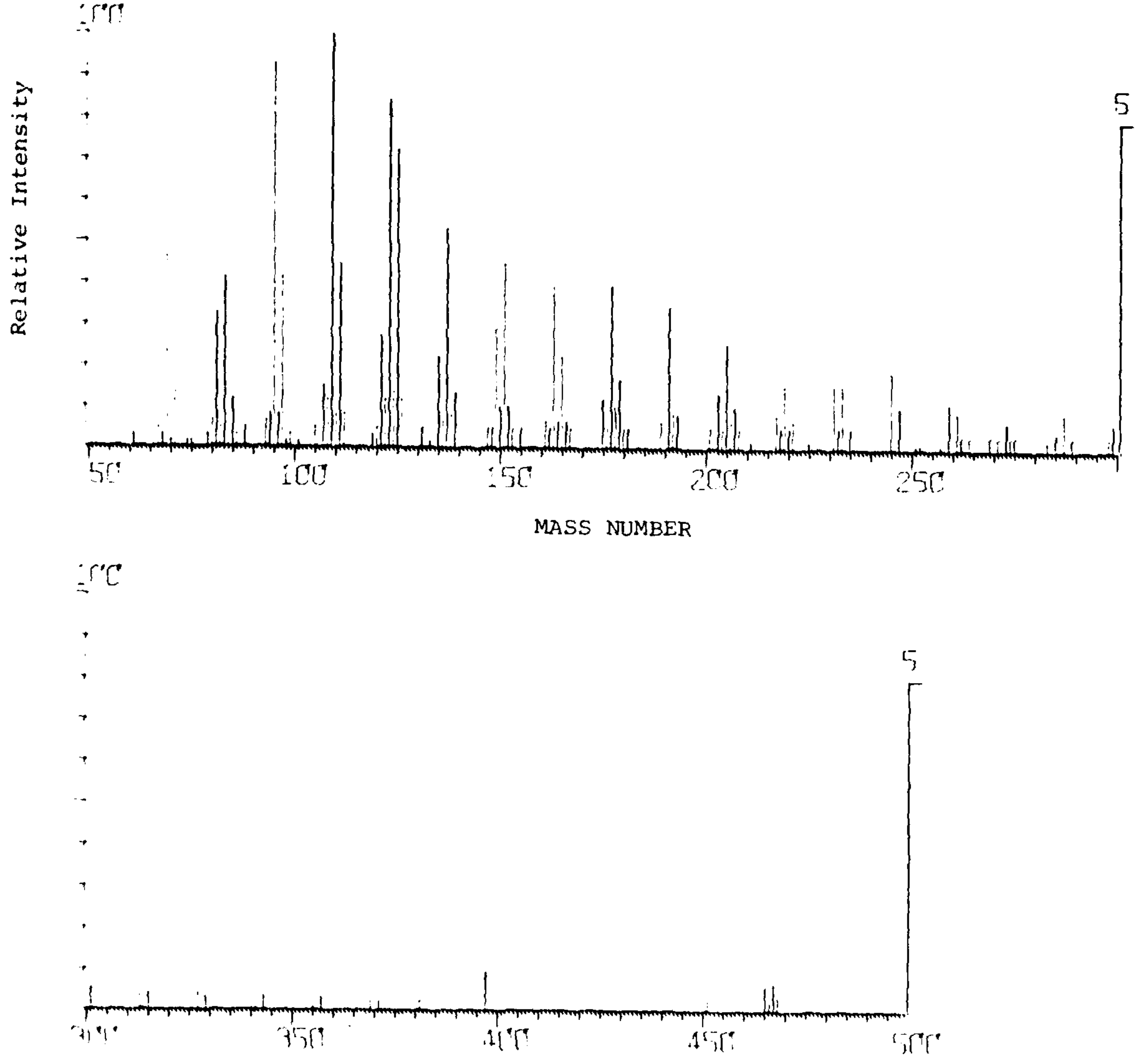
$C_{31}H_{58}$ R.I. 3046

FIGURE 6



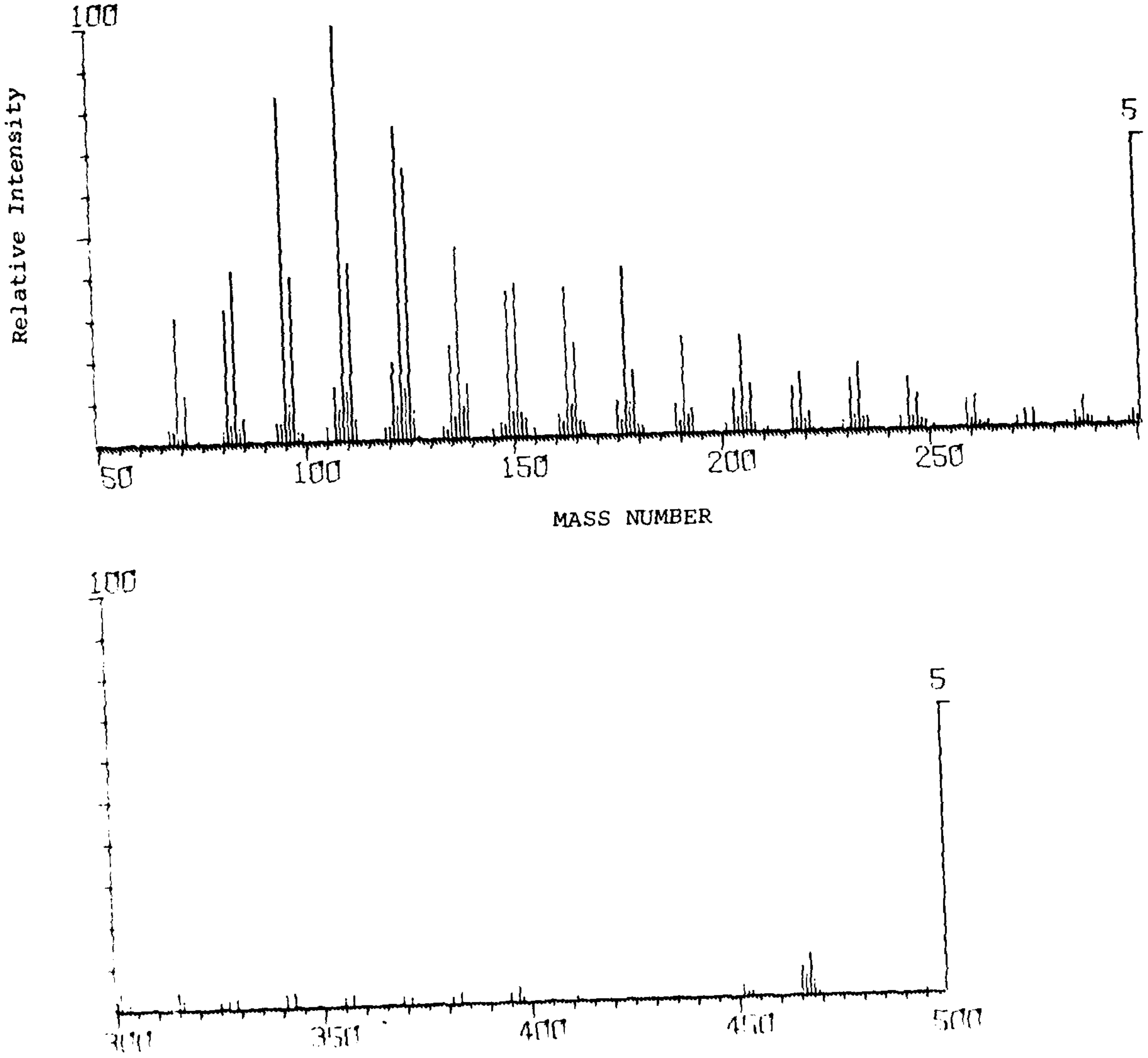
$C_{31}H_{60}$ R.I. 3071

FIGURE 7



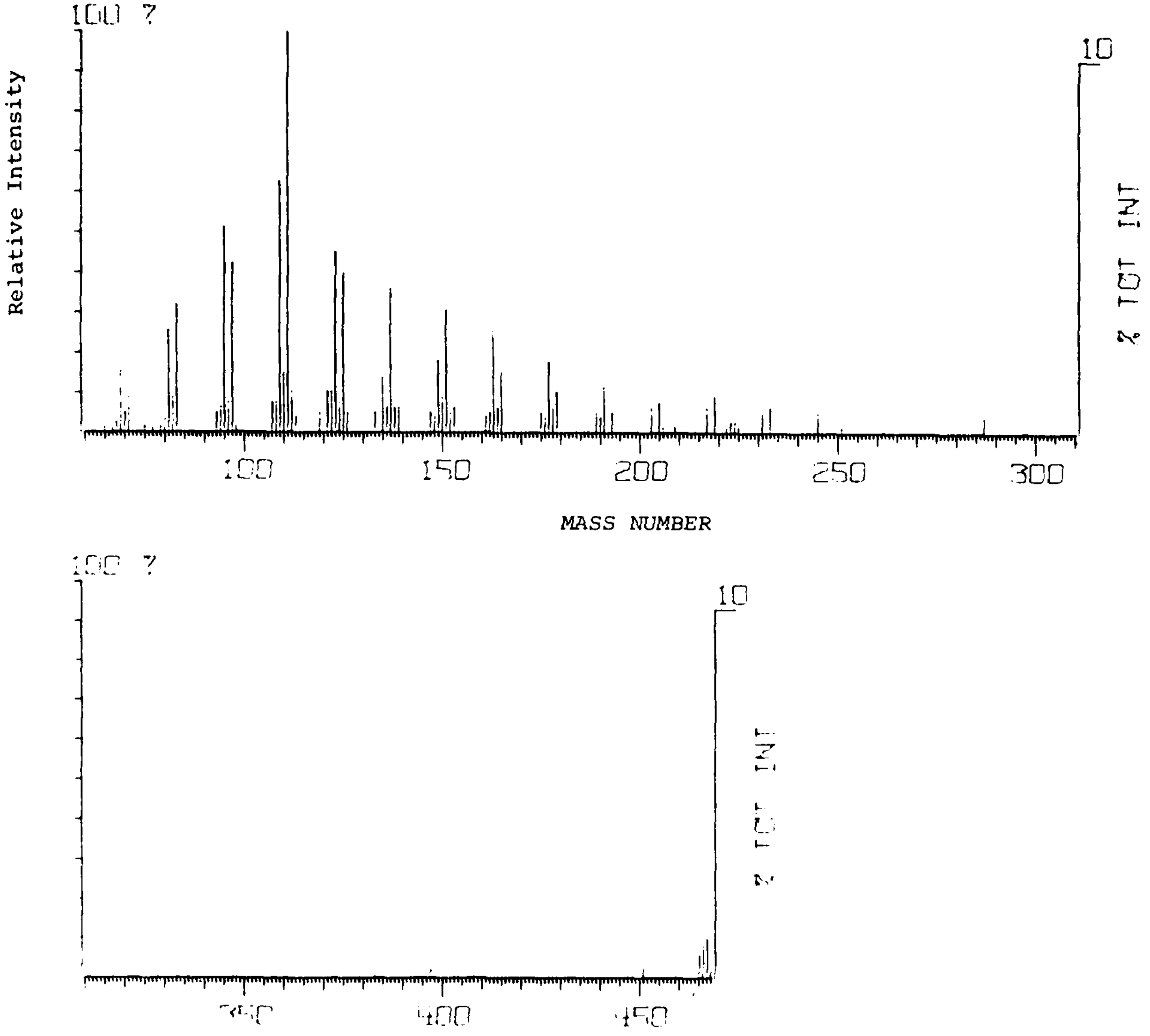
ISOBOTRYOCOCCENE $C_{34}H_{58}$ R.I. 2707-9

FIGURE 8



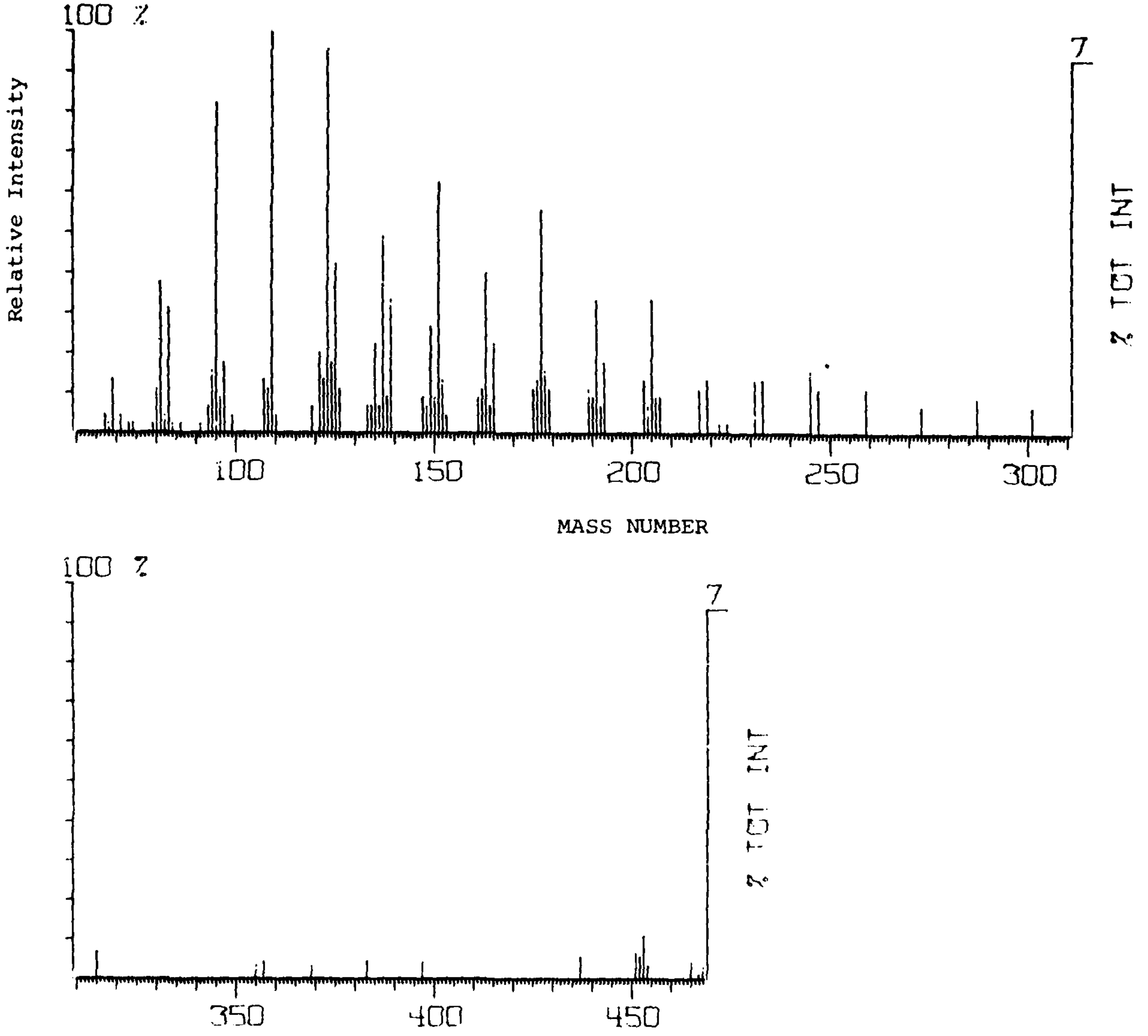
BOTRYOCOCCENE $C_{34}H_{58}$ R.I. 2728-31

FIGURE 9



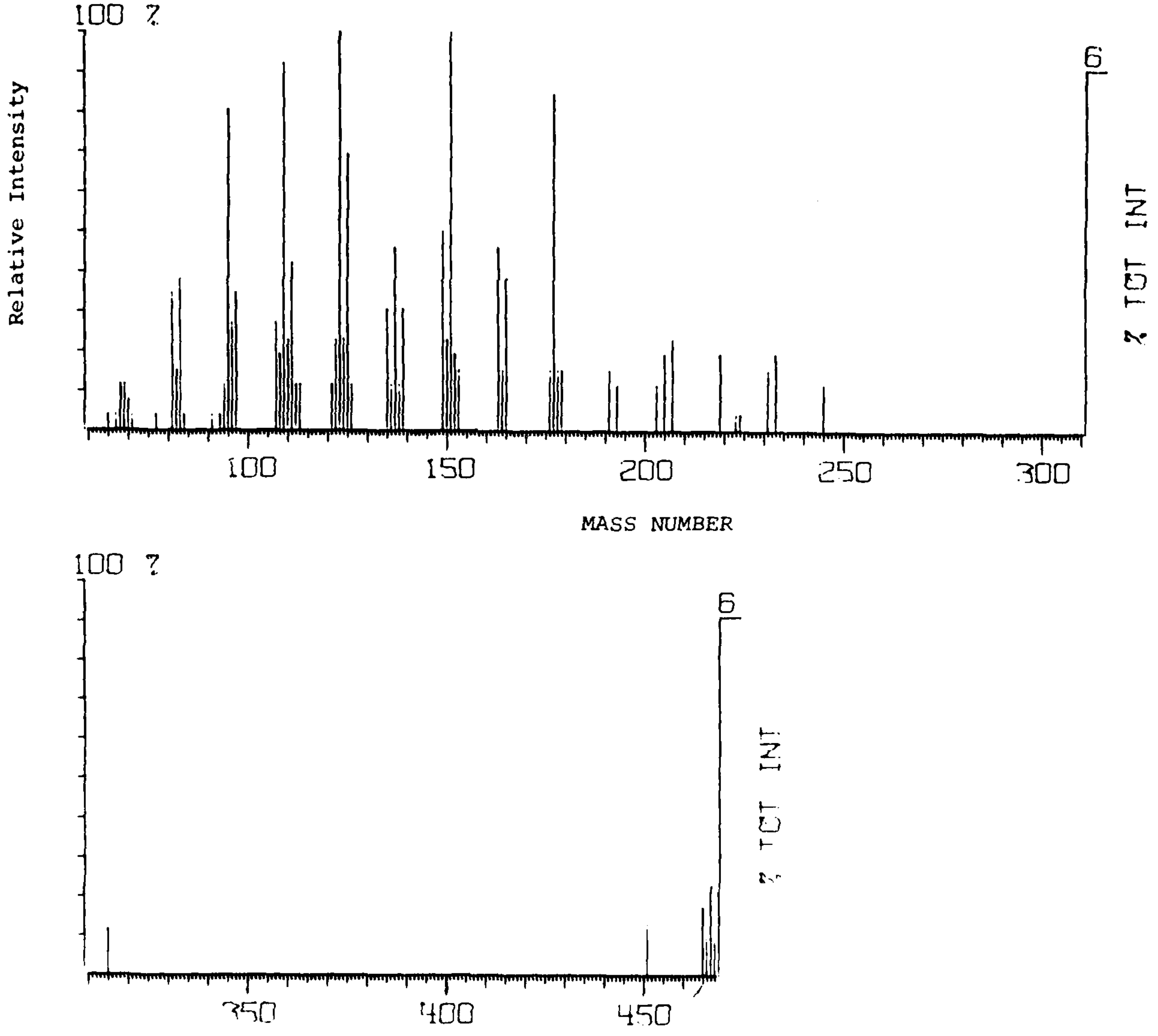
$C_{34}H_{56}$ R.I. 2727

FIGURE 10



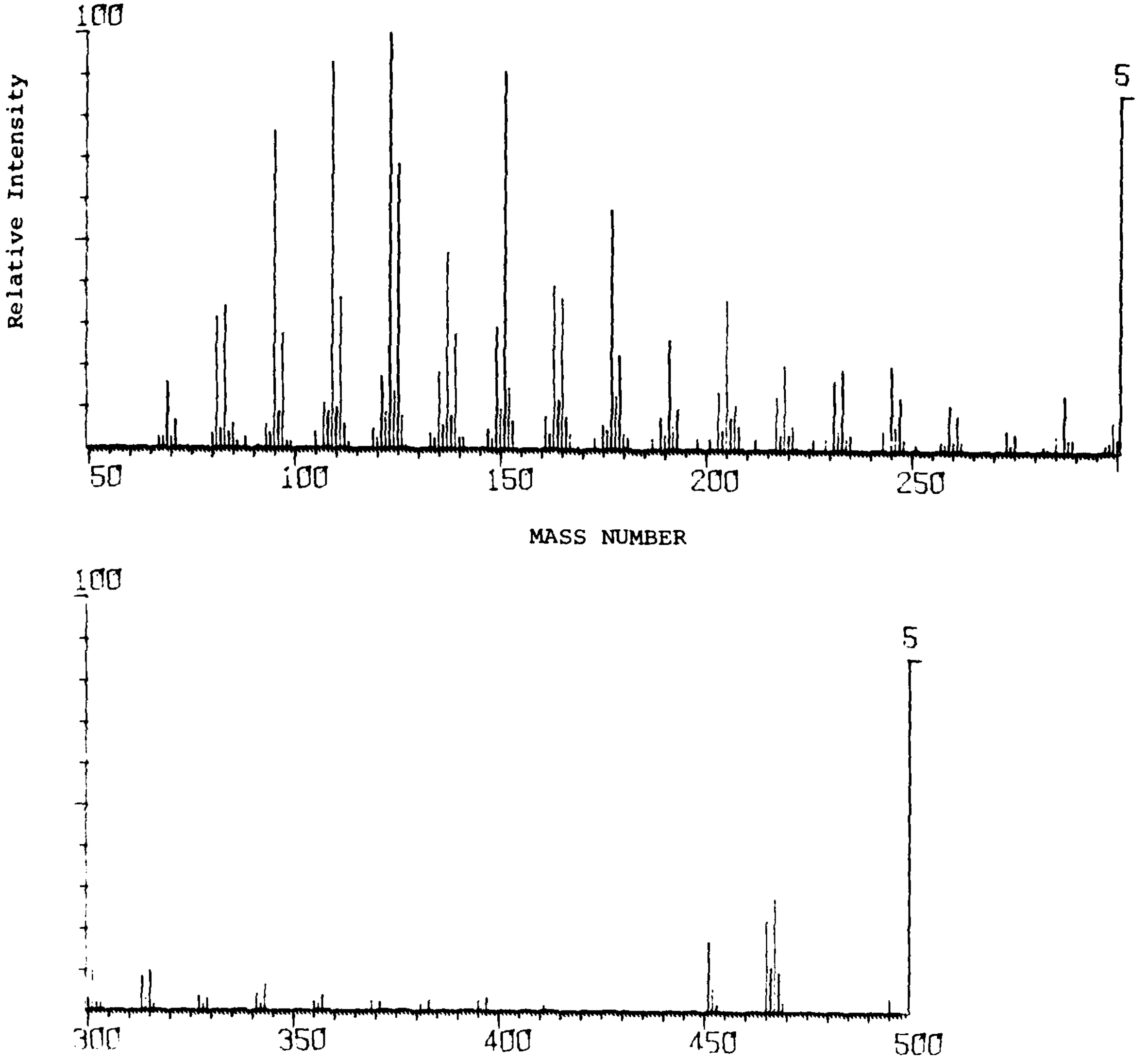
$C_{34}H_{58}$ R.I. 2732

FIGURE 11



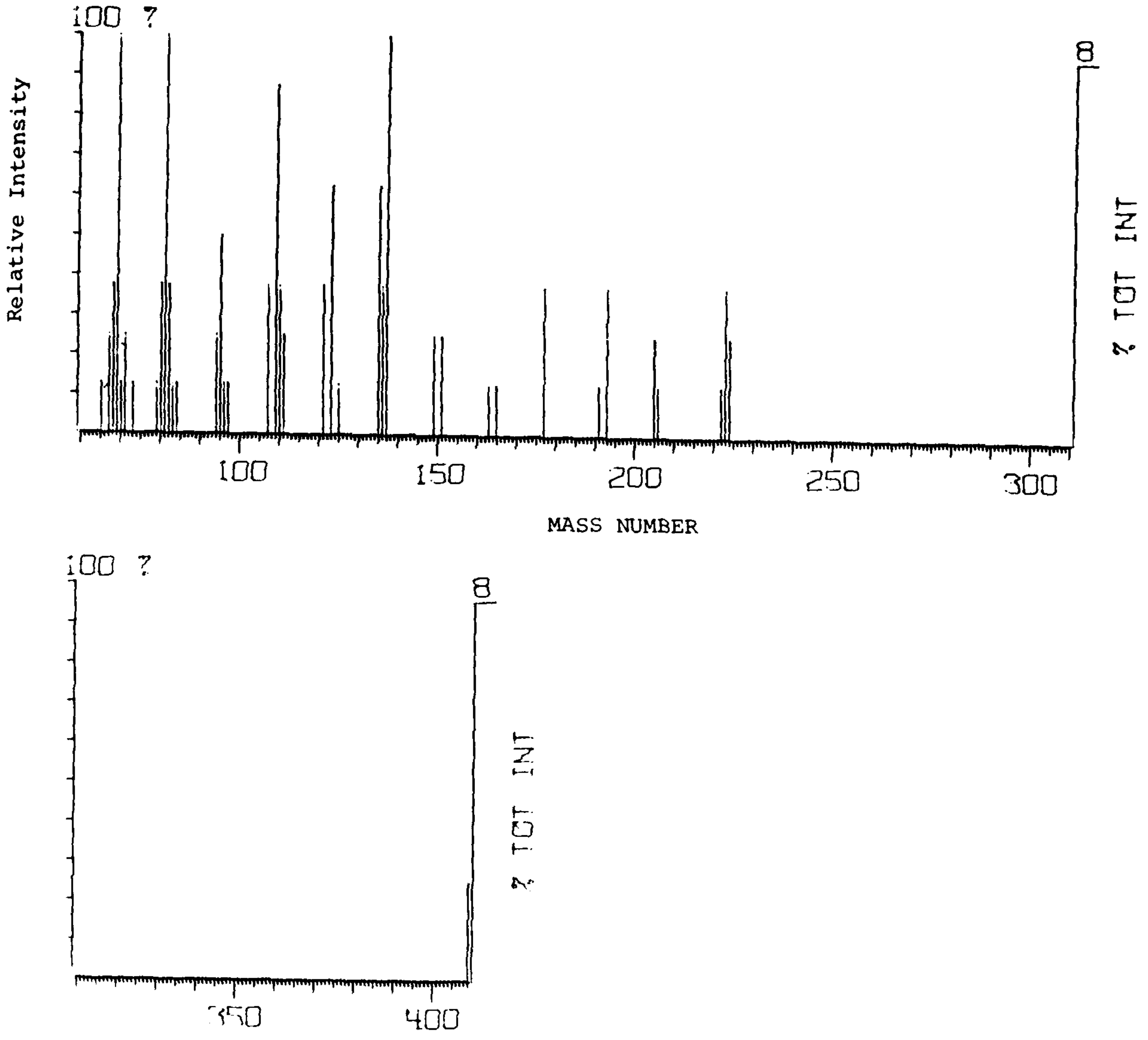
$C_{34}H_{58}$ R.I. 2752

FIGURE 12



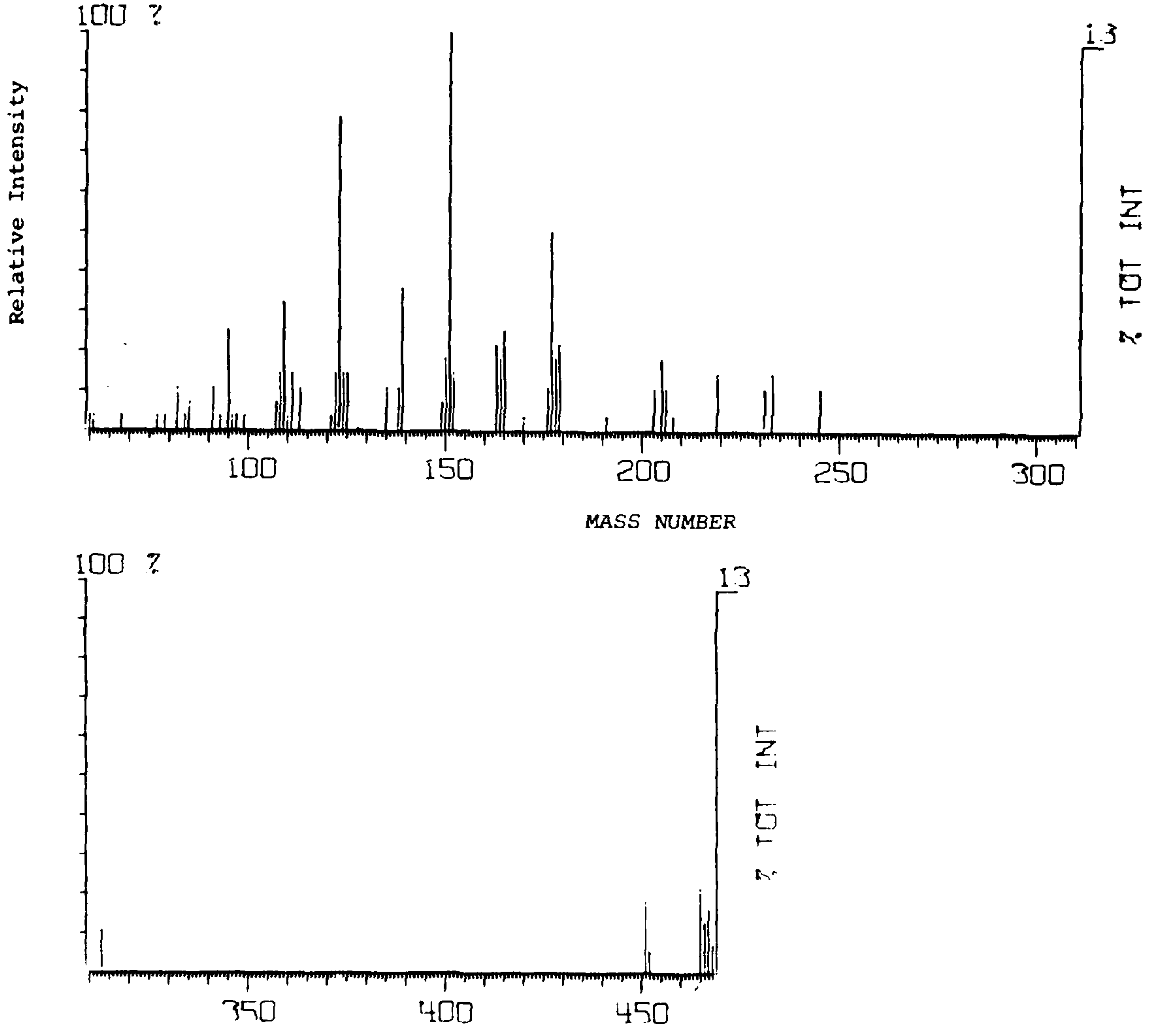
$C_{34}H_{58}$ R.I. 2758-62

FIGURE 13



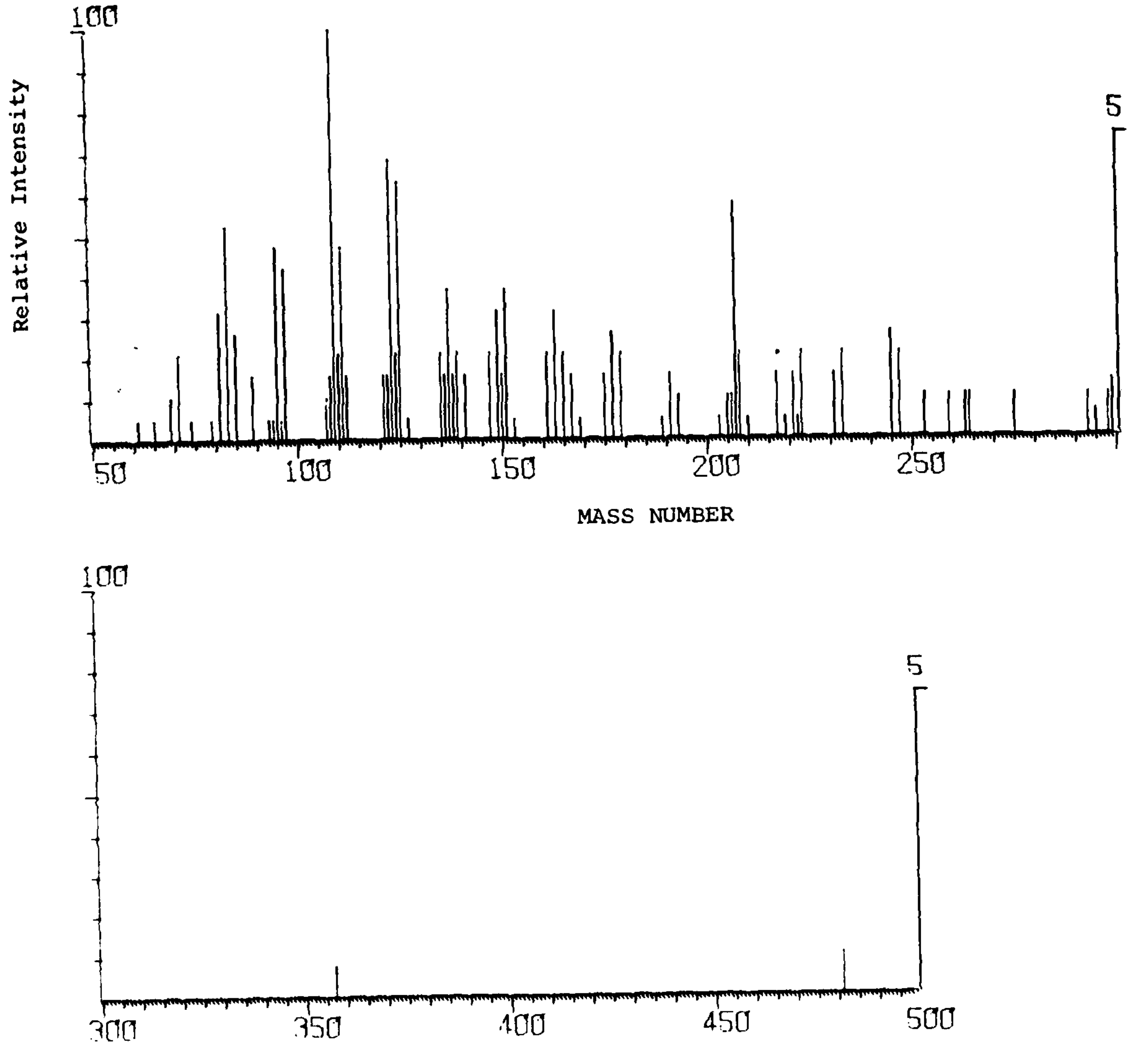
$C_{30}H_{50}$ R.I. 2784-5

FIGURE 14



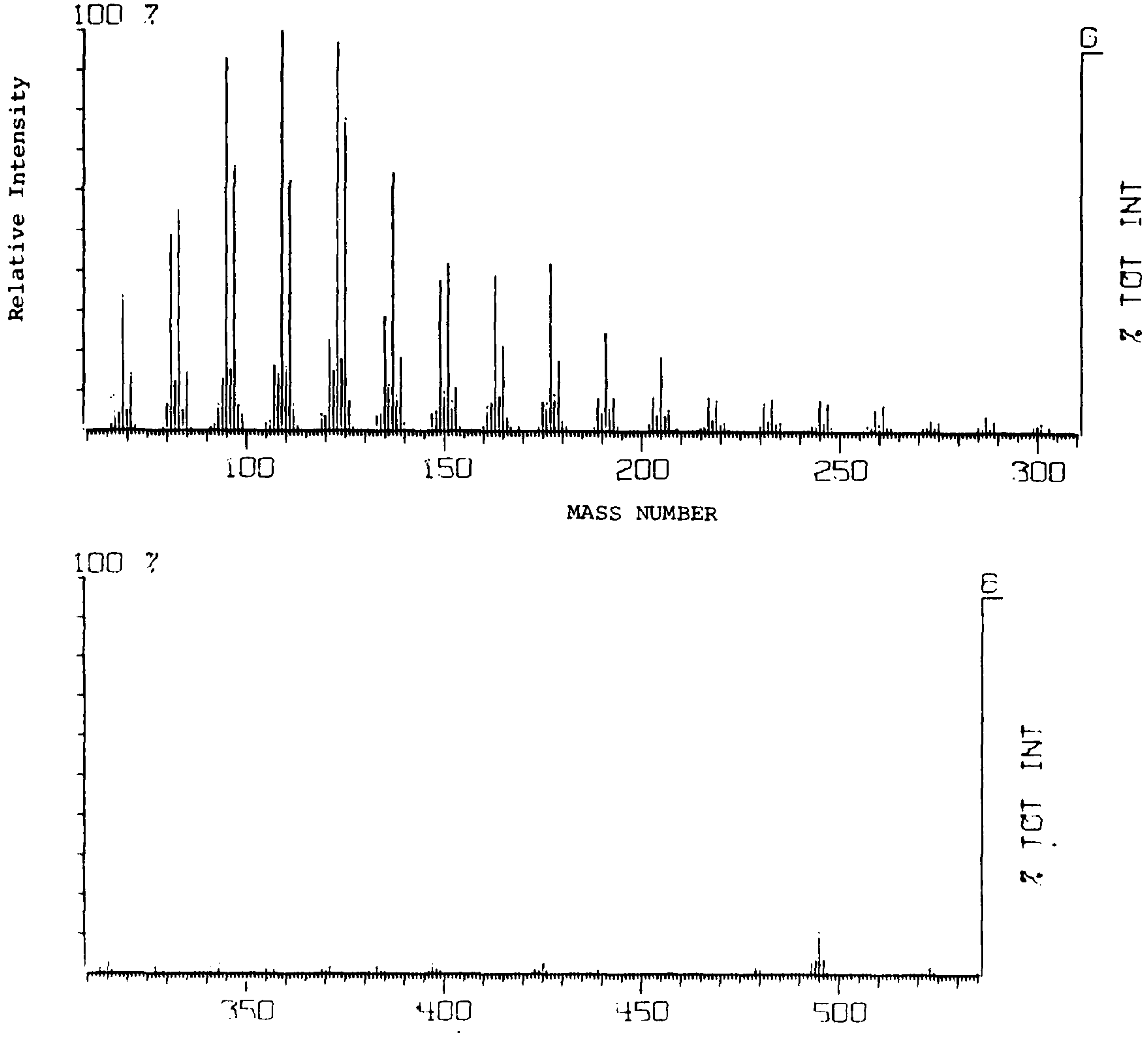
$C_{34}H_{58}$ R.I. 2790

FIGURE 15



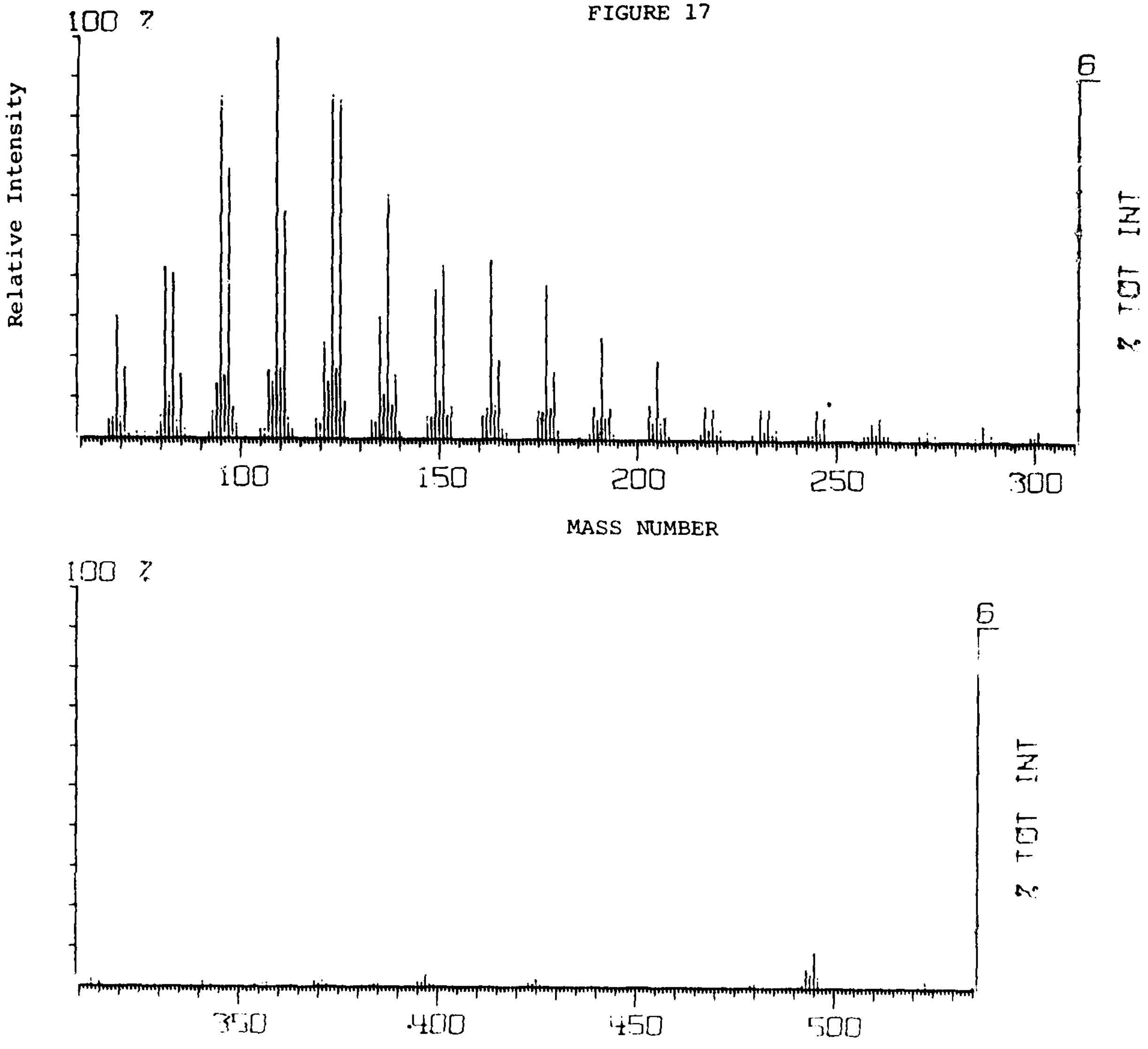
$C_{35}H_{60}$ R.I. 2798

FIGURE 16



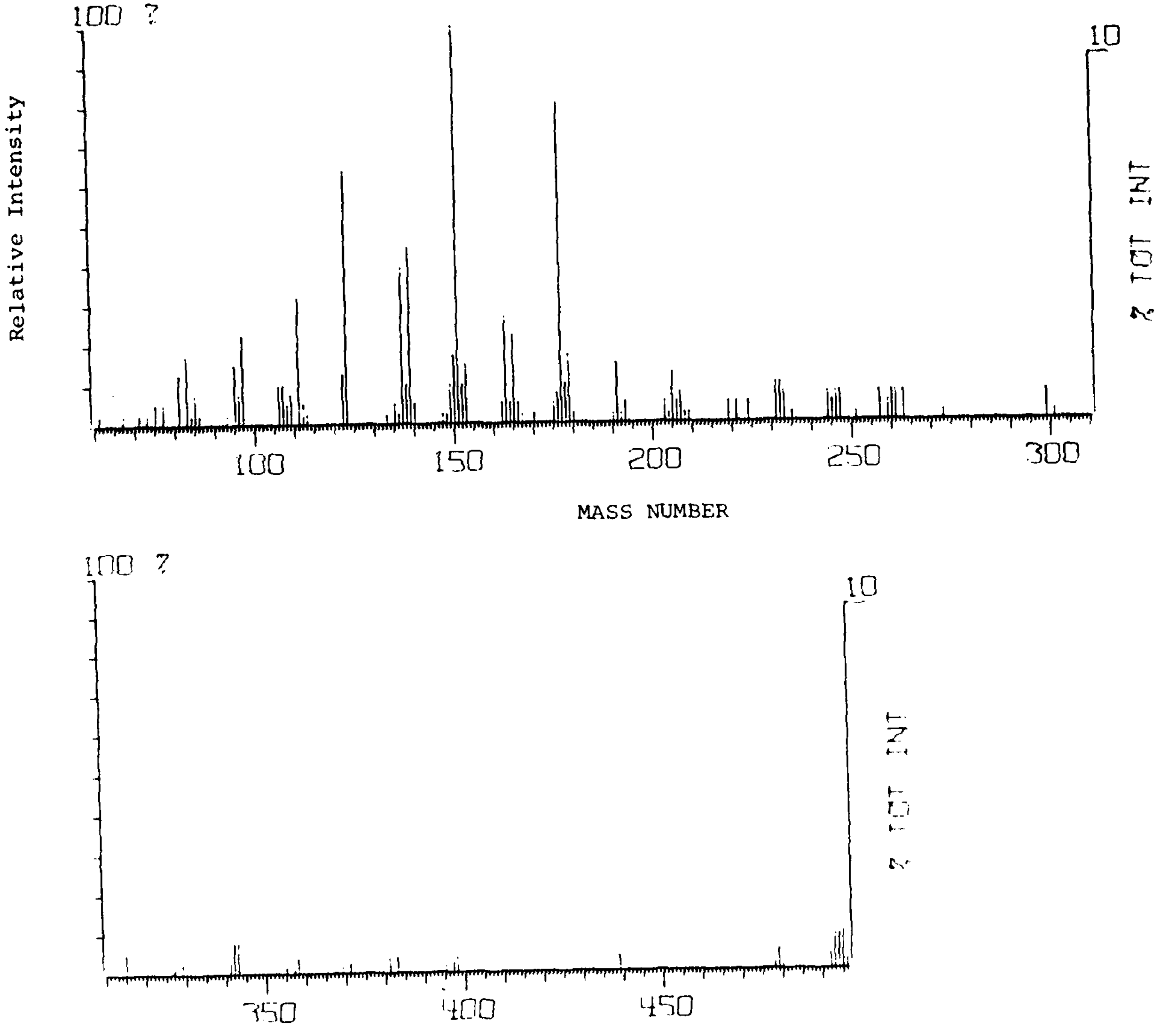
$C_{36}H_{62}$ R.I. 2878

FIGURE 17



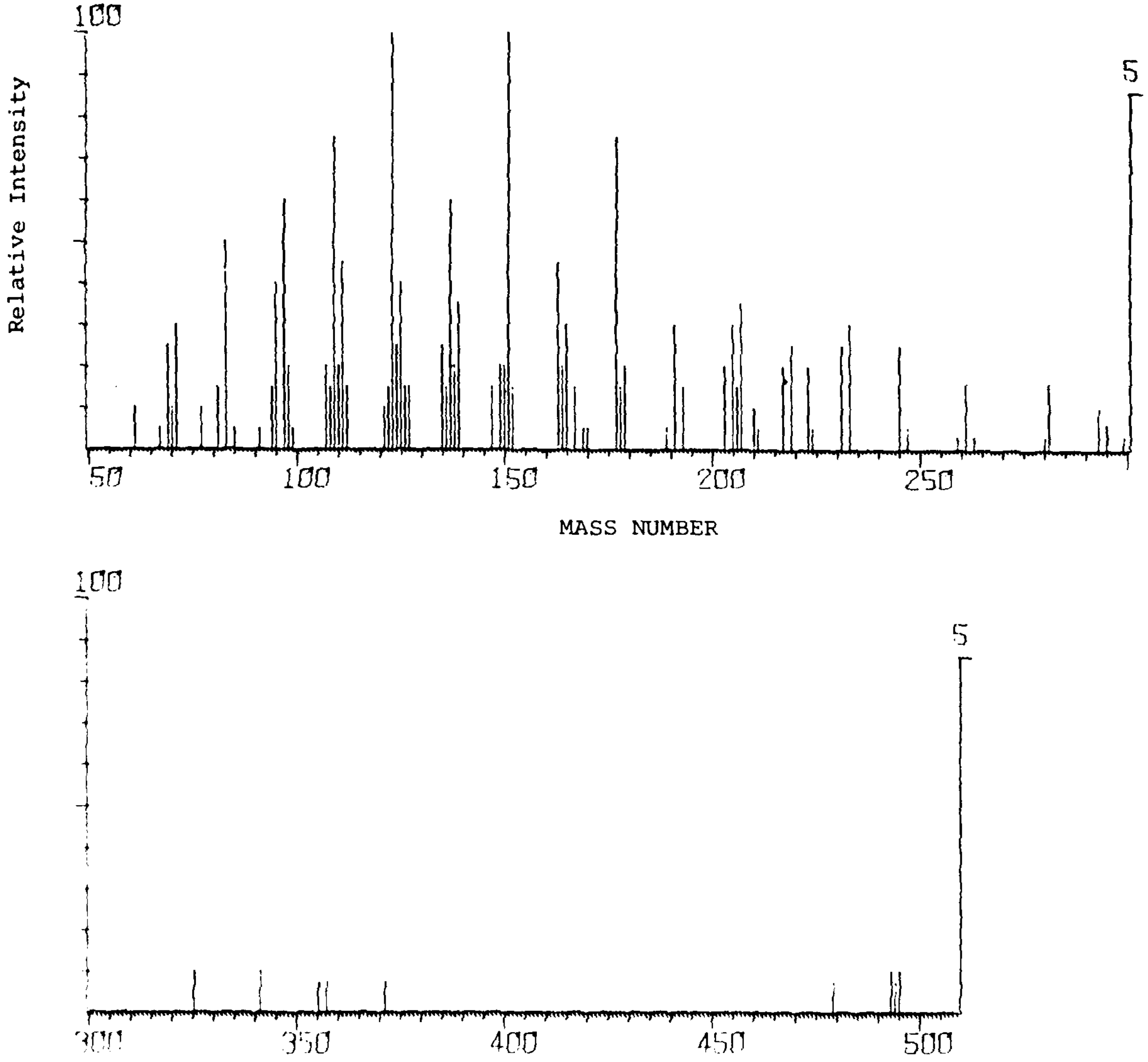
$C_{36}H_{62}$ R.I. 2908-2920

FIGURE 18



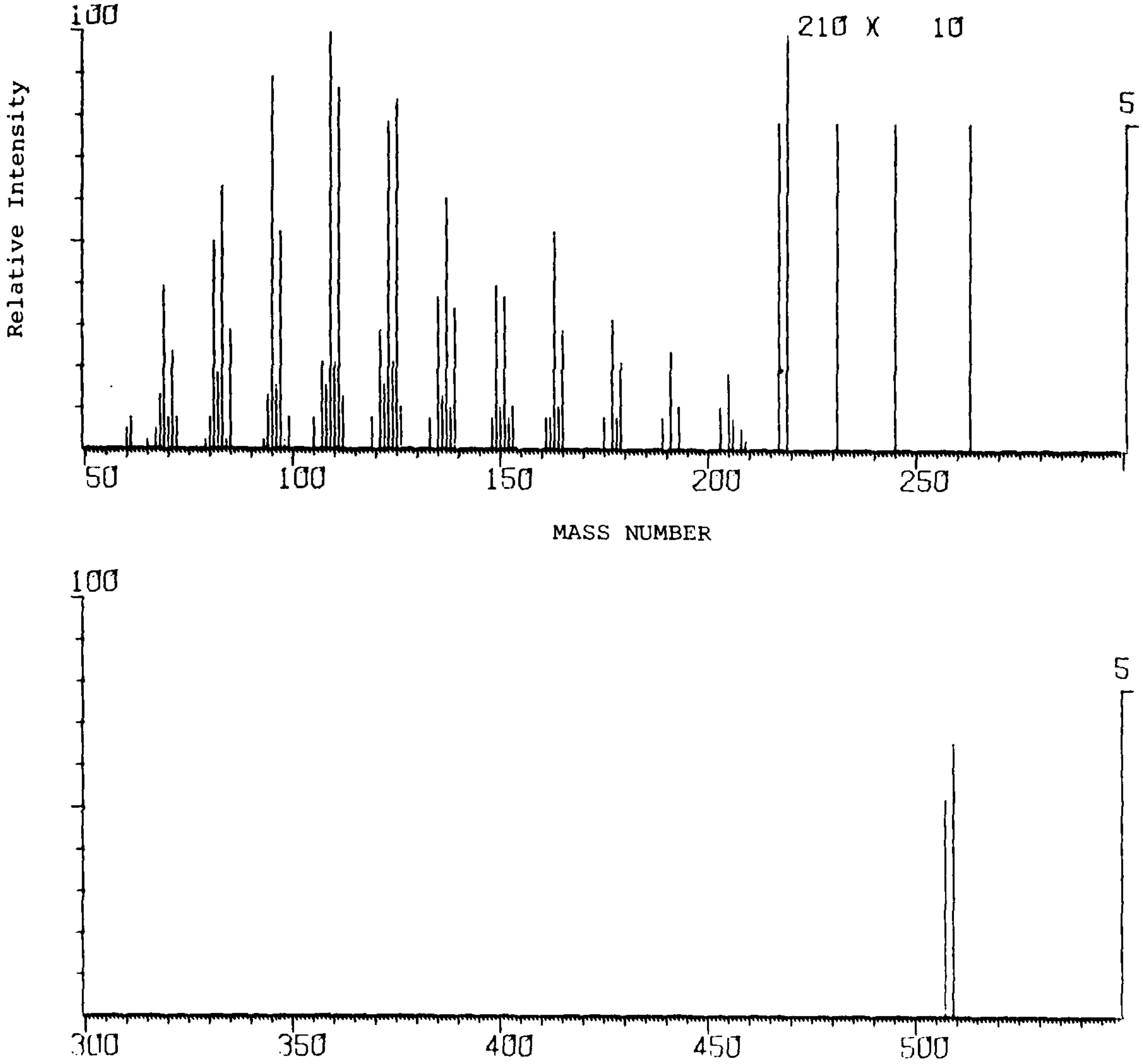
$C_{36}H_{62}$ R.I. 2908

FIGURE 19



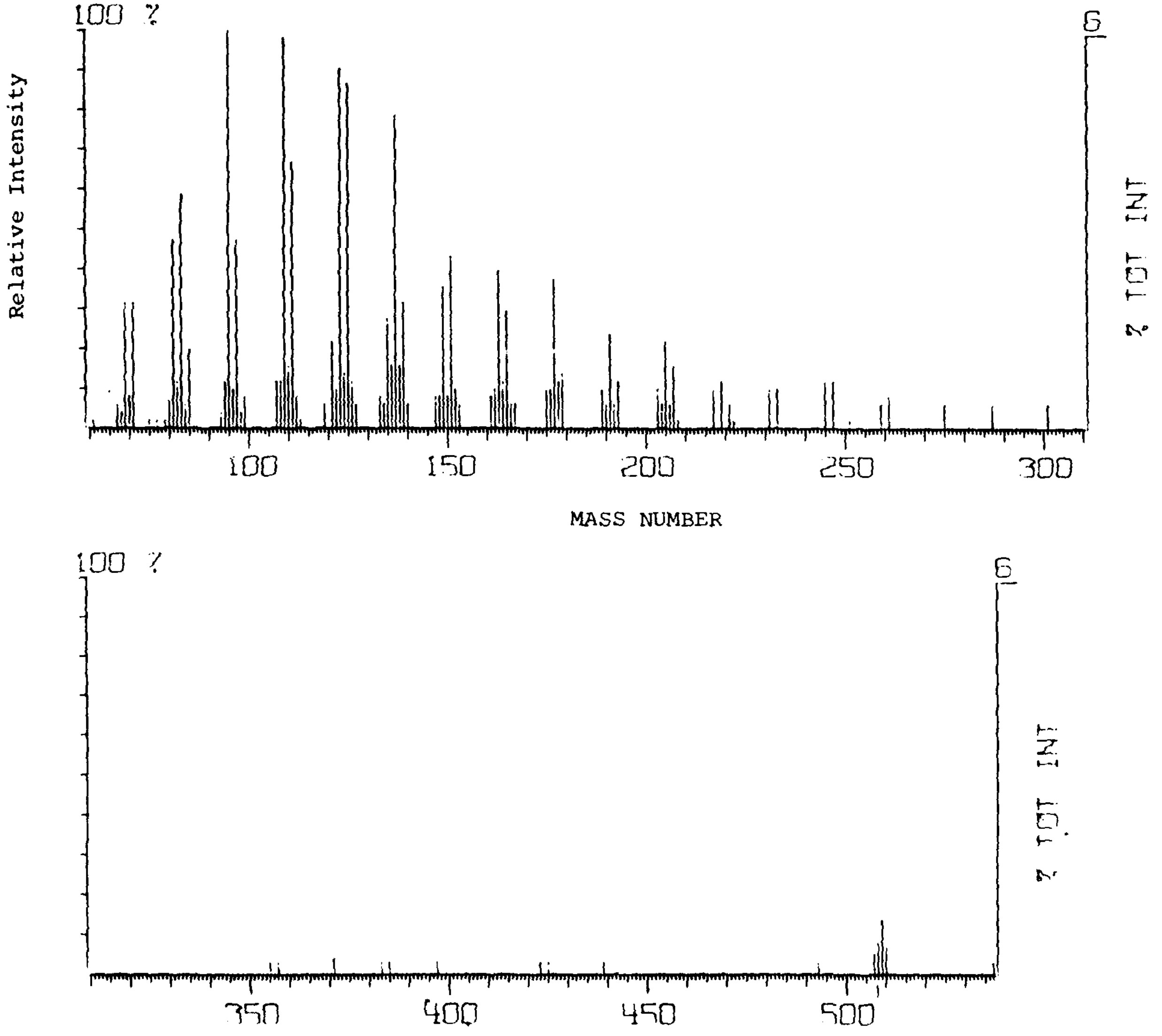
$C_{36}H_{62}$ R.I. 2937

FIGURE 20



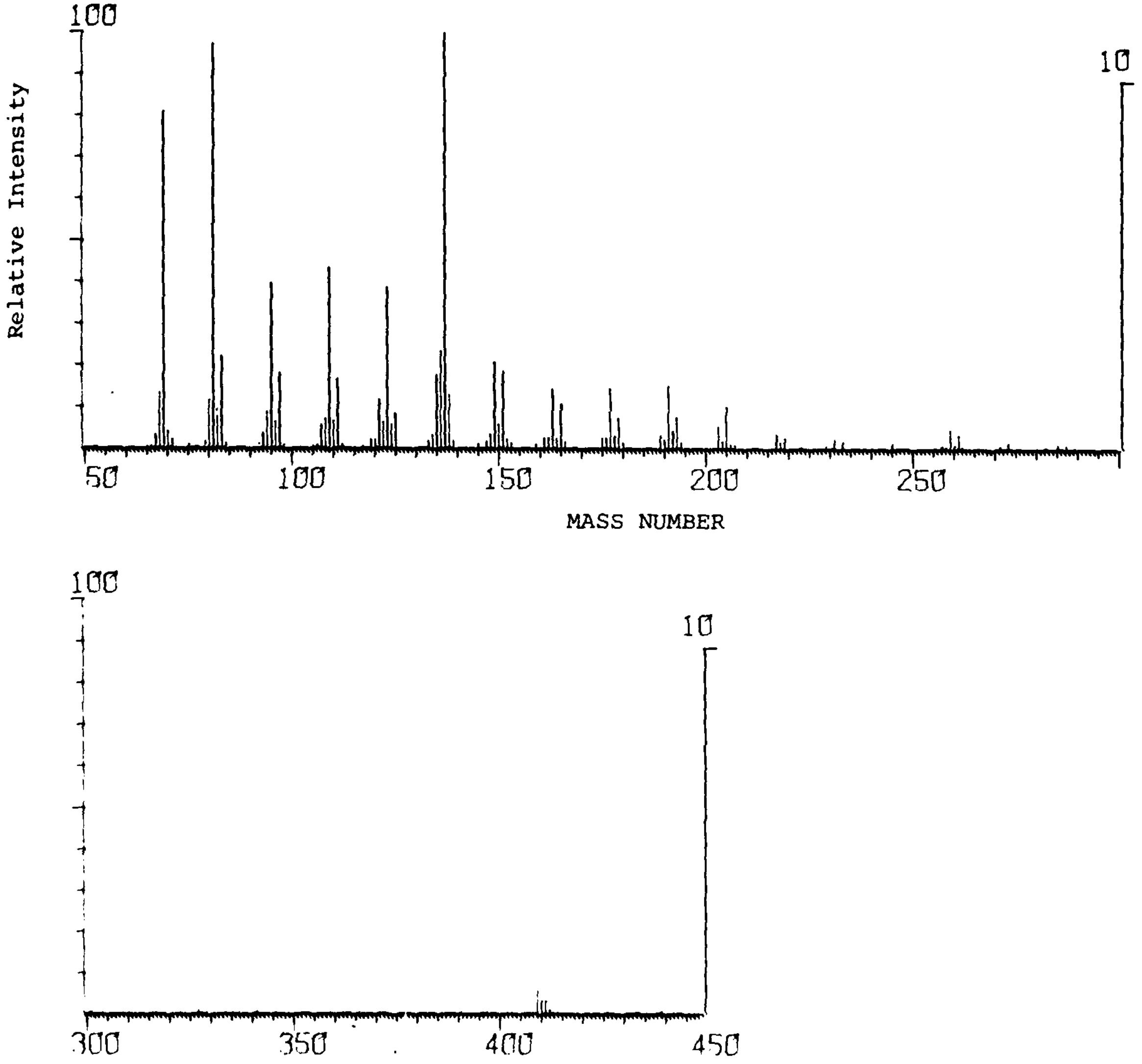
$C_{37}H_{64}$ R.I. 2957-64

FIGURE 21



$C_{37}H_{64}$ R.I. 2974-6

FIGURE 22



SQUALENE $C_{30}H_{50}$

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