CHEMICAL, BEHAVIORAL, AND CHEMOTAXONOMIC STUDIES OF ANTS

by

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CHAPTER I

INTRODUCTION

For centuries the social system of ants has fascinated man. Often, much intelligence and purposiveness of action have been attributed to these insects. Because the coordinating forces behind these social systems were not, in most cases, readily detectable by eye or ear, mechanistic theories to account for this type of behavior had to await the development of sensitive physical instrumentation.

Ants, as well as many other social insects, communicate largely by the release externally of chemicals and the subsequent detection of these chemicals by other ants. Often sterotyped responses are rleased upon detection of a particular chemical. To this end special glands to produce signal chemicals as well as sensitive sensory receptors have evolved. Ants, generally, have a well developed system of exocrine glands which function to release chemicals used for communication and defense (Wilson, 1963).

This dissertation reports on the isolation, behavioral, and chemotaxonomic significance of some chemicals from ants. The major part of this work is concerned

with chemicals which may have significance in the social life of the ants. The remainder of the work is concerned with the use of free amino acids as a possible chemotaxonomic tool. Specifically, this research has four objectives: (1) the isolation, identification, and behavior significance of the alarm pheromone of Pogonomyrmex barbatus (F. Smith), (2) the identification of some hydrocarbons associated with abdominal glands in Novomessor cockerelli (E. André), (3) the value of volatile chemicals from ants as a taxonomic tool, and (4) the usefulness of free amino acids in ant chemotaxonomy.

CHAPTER II

THE ISOLATION, IDENTIFICATION, AND BIOLOGICAL SIGNIFICANCE OF 4-METHYL-3-HEPTANONE FROM POGONOMYRMEX BARBATUS

Goetsch (1951) suggested the presence of chemicals, which when secreted by ant workers, caused alarm and excitement in fellow workers. These chemicals apparently were secreted by abdominal glands in the ants with which he worked. Karlson and Butenandt (1959) have suggested that social chemicals such as these should be called pheromones. Wilson and Pavan (1959) by dissection and bioassay traced the origin of alarm substances in Dolichoderine ants to their specific abdominal glandular origins.

Wilson (1958) reported the presence of an alarm chemical in Pogonomyrmex (Latreille) secreted from the mandibular glands of the worker ants. Brown (1959) likewise found in New World army ants alarm chemicals secreted by the mandibular glands.

As might be expected, most success in pheromone identification has been with alarm substances. This is true because alarm substances are generally produced in larger quantities than other pheromones and usually are

volatile and odoriferous. The chemicals identified, their origin, and behavioral significance have been reviewed by Wilson (1965) and Cavill and Robertson (1965). Since then, other studies have been done by Blum and Warter (1966a), Cavill and Clark (1967), McGurk et al. (1966) and Moser et al. (1968).

Ant alarm pheromones studied to date are not species specific in the sense that chemicals other than the alarm pheromone will release alarm behavior in a given ant species. However, studies by Blum et al. (1966a, 1966b) and Moser et al. (1968) indicate that the quantity of alarm pheromone required to elicit alarm behavior is smaller than the amount of related ketones required to cause the same response.

Measurement of olfactory acuity in insects is most conveniently based on behavioral thresholds. However, the minute quantities of chemicals involved as well as the natural variation in the insect response, make behavioral thresholds difficult to determine. Although Bossert and Wilson (1963) have calculated a theoretical alarm threshold for P. badius, the alarm threshold has been determined experimentally for only one species of ant (Moser et al., 1968).

This chapter reports on the identification of 4-methyl-3-heptanone from Pogonomyrmex ants. The function,

specificity, and threshold concentration for 4-methyl-3-heptanone was determined for these ants.

Experimental Methods

Ants used in this study were obtained by two methods: by collecting ants from wild nests or by collecting mated females after a mating swarm and allowing them to establish colonies in laboratory nests.

Ants were collected conveniently by pit trapping with gallon jars placed into holes dug into the ant nest. To prevent escape of the ants, scentless talc was powdered on the sides of the jar. A vacuum type insect collector was also used (Hall, Drew, and Eisenbraun, in Press).

Colonies were established by collecting fertile queens immediately after their mating flights. The best time for collection is generally following a rain in July and August, and in this area, it is possible to pick up several hundred queens in a day following such flights. The queens are usually captured while on the ground searching for a nesting place.

The captured queens were individually housed in nests constructed of one to several standard plastic dishes connected by one-fourth inch diameter plastic tubing. One petri dish (the brood chamber) contained a 0.25 in. layer of plaster of Paris occupying about one third of the floor area. A 0.25 in. diameter plastic

tube was inserted through a hole in the top of the brood chamber into the plaster of Paris. This tubing provided a convenient means of moistening the plaster of Paris. A second dish attached to the brood chamber served as a foraging chamber. Other dishes were provided for colony expansion, burial grounds, and refuse heaps. Nests of this type have been previously described by Forest (1962) and Ettershank (1965). The nests were usually shelved in such a manner that the brood chamber remained dark and the foraging chamber was exposed to light.

One colony of ants was allowed to forage in a large forage arena constructed of plywood. The arena was 5.5 ft x 2.5 ft x 3 in. An electrically heated nichrome heating wire at the top of the side walls prevented the escape of the ants. The wire was insulated from the plywood by a band of asbestos and heated to about 135^{o} C by a powerstat. The plastic nest sat outside the foraging arena. It was connected to the foraging arena by tygon tubing leading into the foraging area through holes in the side wall beneath the heating wire.

The ants were fed German millet, hamburger meat, and fresh insects when available. The rearing system used required that the colonies be cared for about three times each week.

A special digging chamber was used to study the digging behavior of the ants. It consisted of a round

finger-bowl upper chamber, 4 in. in diameter and 2 in. deep, and a 1 in. deep, 1 in. diameter subchamber opening directly into the upper chamber through the bottom of the upper chamber. The floor of the upper chamber was covered with nylon gauze to close the opening between the chambers and the bottom of the upper chamber was covered with one centimeter of sand. A glass tube attached to the side of the subchamber served as a port to introduce ants or chemicals into the subchamber. Ants were introduced into the upper chamber by connecting the upper chamber into the tubing system of the nest via glass entrances on either side of the upper chamber, or ants could be placed directly into the upper chamber.

Two methods were used in an attempt to determine the selectivity of 4-methyl-3-heptanone in releasing alarm behavior in Pogonomyrmex barbatus (F. Smith). The first was a modification of the method used by Bossert and Wilson (1963). The ketones tested were either obtained commercially or were synthesized from commercially available chemicals. When necessary, chemicals were purified by preparative gas chromatography. All chemicals were of at least 95-98% purity as shown by gas chromatographic analysis.

Glass tubes, 3 ft long and 1.25 in. inside diameter, had a wire screen barrier fused into the glass 5.5 in.

from one end. Corks covered with aluminum foil were used to stopper both ends of the tube. About 75 ants anesthetized by CO_2 were placed in the tube and allowed 2 hours to become accustomed to the tube. One tenth microliters of the chemical to be tested were placed on a disc of filter paper, 1 cm in diameter, which was then immediately placed in the screened end of the tube. The tubes were quickly restoppered. The time required for the ants to first respond to the chemical and their distance from the source were recorded.

Two tests were usually made each day, a test before noon and the other in the afternoon, with about 3 hours between tests. The ants were collected from "wild" colonies near Stillwater, Oklahoma and kept in laboratory petri dish nests between tests. The ants were not used for more than two days of testing. Tests were replicated 9 times, with random selection of 10 treatments being a normal test.

The parameter estimated was the square root of time/distance of the variance for a completely randomized experiment as described by Steele and Torrie (1960).

The second method used for the testing of the efficiency of different ketones in producing alarm behavior was the air stream dilution method used by Moser et al. (1968). As with the procedure described immediately before this one, all chemicals tested were at least 95-98% pure.

The chemical to be tested was introduced in excess into a closed flask and the air above the liquid in the flask was allowed to become saturated with the vapor. This vapor was drawn from the closed flask with a 10-ml glass syringe and injected into the test chamber either undiluted or serially diluted with air. The vapor was introduced into a stream of air flowing at 25 ml/min into a 6 liter canister used as a foraging arena by a colony of Pogonomyrmex ants. The vapors were removed after each test by purging the canister for 20 minutes with an air stream drawn by an aspirator. The tests were conducted in an air-conditioned room at 24° C. The test was considered positive when unoccupied workers were observed to lift their heads with antennae waving about and pointing almost straight upward. This behavior was followed by attempts to climb the sides of the test canister.

Because of the inherent subjectivity in determining exactly when alarm behavior was exhibited, it was felt that attempts to test dilution levels at less than a power of ten would be meaningless. The behavioral threshold concentration, K, may be estimated from the dilution data using the known vapor pressure of several of the ketones tested (Jordan, 1954). Because the vapor pressure of closely related chemicals is similar, in

those cases where the vapor pressure of a chemical was not known, the value for a closely related chemical was used.

The K value was determined as follows: The molar concentration of vapor in saturated air is given by the formula $n_c = VP_c/RT$ where V is the volume of the container from which the vapor is drawn, 265 ml in this case; P_c is the vapor pressure of the chemical in mm Hg divided by the atmospheric pressure in mm Hg; R is the gas constant, 82.054 (ml atm per mole degree), and T is the absolute temperature at which the experiment was conducted, 297° K. N_c, the concentration in moles/ml of chemical in saturated air, is multiplied by Avogadro's number, 6.023×10^{23}, and the resulting value is divided by the highest dilution which elicited a behavioral response. The resulting value is the threshold concentration in molecules per ml of air. The concentration in the six liter test canister can only be estimated. A baffle was installed directly below the air inlet to prevent downward streaming of the gas sample, but the shortness of the test (about 25 sec.) did not give enough time for equilibrium to be established in the chamber. The average concentration in the test chamber was estimated at 3×10^3 following the suggestion of Moser et al. (1968). The accuracy of this estimate has a direct effect on that of the K values but does not

affect the ranking of chemicals according to effectiveness in eliciting alarm behavior.

Dissections were performed with watch maker's forceps and iridectomy scissors under a dissecting microscope. The nomenclature of Herman and Blum (1967) was used in naming the glands. The glands were either heated in a pyrolysis loop and their vapors directly introduced into the gas chromatography column or the glands were extracted with ether before chromatography.

Results

For the identification of the unknown alarm substance, 700 grams of ants were collected, which were killed by freezing and stored frozen until used. The ants were homogenized in a Waring blender and the volatile components were obtained by steam distillation. After saturation with salt, the steam distillate was extracted with ether and the extract concentrated to 2 ml by distillation through a spiral column. This extract showed eleven peaks when analyzed by gas chromatography (0.25 in. x 10 ft column of 25 per cent Carbowax on acid-washed Chromosorb W at 130^{o} C - helium flow rate, 52 ml/min; Figure 1). Peaks 1 and 7 are at minimum recorder sensitivity. The remaining peaks are set 20

times recorder sensitivity of peaks 1 and 7. The extract yielded 150 microliters of the pure unknown alarm substance.

Mass spectrometry and nuclear magnetic spectroscopy were used to identify peak 7 as 4-methyl-3-heptanone. The mass spectrum showed a molecular ion at mass 128 with characteristic fragmentation peaks at m/e = 57 (loss of the larger alkyl group by α-cleavage, m/e = 86 (β-cleavage), and m/e = 71 and m/e - 29 (the latter two ions resulting from α - cleavage on either side of the carbonyl with the positive charge remaining on the alkyl group) (Budzikiewcz et al., 1964). Although the low-resolution mass spectrum gives the molecular weight and an indication of the types of functional groups present, it does not provide positive identification of such compounds unless the spectrum of the authentic compound is available. This is because several 8-carbon ketones have the same molecular weight and some of them give similar fragmentation patterns.

The NMR spectroscopy showed absorption for three protons α to a carbonyl group consisting of a quartet with an underlying multiplet centered at 2.43 δ . This is characteristic of an ethyl ketone with brancing on the opposite side of the carbonyl group. The only 8-carbon ketone to fit this spectrum well is 4-methyl-3-heptanone.

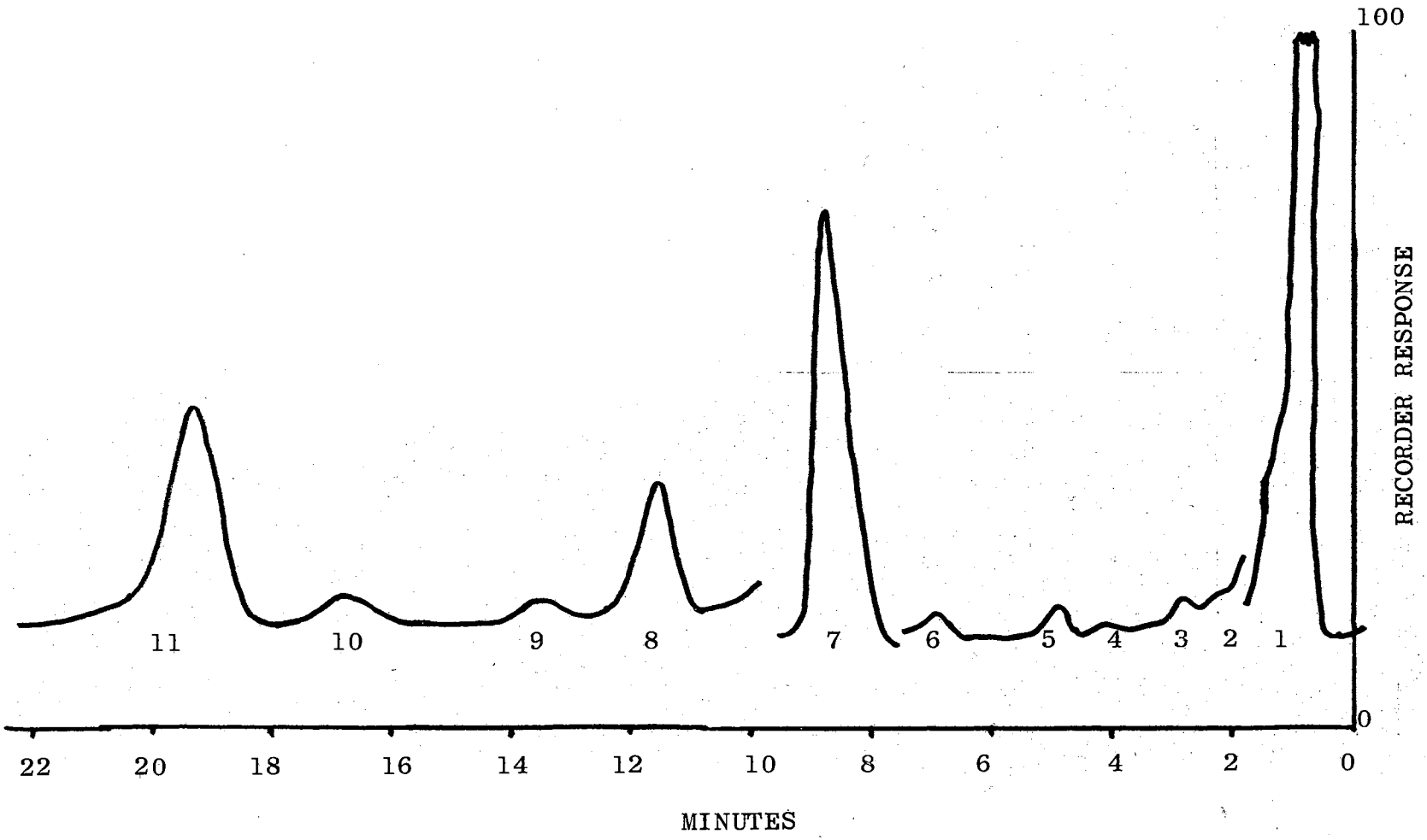

Figure 1. Gas Chromatogram of P. barbatus Ether Extract. See text for conditions and column.

Positive identification was confirmed by comparison of NMR and mass spectrum with those of the authentic ketone.

The ketone, 4-methyl-3-heptanone, comes from the mandibular glands of P. barbatus. The glands isolated by dissection were placed in the closed pyrolysis loop of a valved gas chromatograph (Instruments, Inc. Model 393). When the compounds which were volatilized by heating the loop to 200° C were diverted into the column, a peak corresponding to 4-methyl-3-heptanone was obtained. A peak indistinguishable from this may be obtained from a single head of P. californicus (Buckley), P. desertorium, Wheeler, P. occidentalis (Cresson), P. commanche, Wheeler, P. rugosus Emery or P. badius.

This ketone apparently releases alarm behavior in P. barbatus. The ants were at first attracted, as observed by Wilson (1958), to a drop of the ketone allowed to evaporate in the nest. As it evaporated, they became alarmed and appeared to release the alarm substance themselves. Twenty microliters of 4-methyl-3-heptanone, evaporated in a 5 ml syringe and injected into a 3 inch diameter, covered, petri dish nest, produced alarm behavior in the ants for 15 to 20 seconds. They quickened their movements, periodically raised their heads high above their bodies, and opened their mandibles. Occasion-

ally they bit and stung debris in the nest. With injections of 1 to 5 microliters, the alarm behavior intensified, and often lasted for more than a minute.

Like army ants (Brown, 1959), P. barbatus workers attacked crushed heads of other workers of the same colony. Crushing the abdomen or thorax of an ant had no effect on the other ants. When a small drop of 4-methyl-3-heptanone was placed on a live, active ant in a colony, the ant was attacked even more readily than the carcass of one with a crushed head. Ants which passed within 3 or 4 cm of the treated ant immediately chased and attacked it, with sometimes as many as three or four trying to bite and sting it at the same time. Often those ants which had contacted the treated ant, and therefore had acquired the odor of the chemical, were also attacked. P. barbatus workers attacked objects such as wads of cotton previously treated with 4-methyl-3-heptanone.

After exposure of a few minutes to this ketone, ants were observed carrying pebbles in their mandibles and infrequently making digging movements with their legs. Wilson (1958) reported similar behavior in P. badius and suggested that ants buried by a cave-in might release a chemical to signal for rescue by nestmates. P. barbatus workers quickly dug out others which were buried in a few cm loose soil. However, injecting 1 to 5 microliters of

4-methyl-3-heptanone beneath a cm of soil or burying pieces of filter paper spotted with 1 microliter drop of this compound, did not initiate digging behavior.

An interesting behavior was noted after prolonged exposure of the ants to 4-methyl-3-heptanone. The ants habitually piled soil and pebbles around the plastic tubing entrance to their nest to form a cone. After prolonged disturbance, the ants used the pebbles to close the entrance to their nest. This behavior was also noted at night after the ants quit foraging.

Markl (1965) showed that stridulations produced by buried workers function as a distress signal among Atta cephalotes L. In an attempt to determine if the digging behavior observed in P. barbatus after the exposure to 4-methyl-3-heptanone was functional or a displacement behavior due to overexposure to the alarm chemical, the digging chamber previously described was used. With this, it was ascertained that ants will dig up buried workers as well as microliter quantities of 4-methyl-3-heptanone.

The alarm behavior is not specific for 4-methyl-3-heptanone. The behavior is released by many volatile chemicals including acids, ketones, and hydrocarbons. These observations have been borne out by other workers (Wilson, 1963; Moser et al., 1968).

Experiments were undertaken to determine if there was an quantitative difference in the quantities of substances needed to release alarm behavior in P. barbatus.

The first experiment was a modification of the method used by Bossert and Wilson (1963). In their experiments they dealt with an unknown chemical for which a diffusion coefficient was estimated. In actuality, the diffusion coefficient is extremely difficult to determine experimentally. If the diffusion coefficient were known, the exact threshold concentration in molecules per cubic centimeter could be determined.

Although, the diffusion coefficients were not known for the chemicals being used, the close simularity between the molecular weights and class of chemicals suggested that one could safely conclude that the diffusion coefficients would be similar. Thus, any differences in reaction time of the ants to different chemicals might be attributed to different threshold concentration values. Since the distance of the ants to the source varied from test to test, the parameter $\frac{T}{D^2}$ was measured, when T was the elapsed time from introduction of the chemical sample to the time the ants reacted and D was the distance of the reacting ants from the source. The tests were run nine times for each chemical, with the results being averaged.

To demonstrate that comparable values were obtained from ants reacting at different distances from the source, more than one observation was recorded for many of the tests as the ants reacted at more remote distances from

the source. Sixty-one such observations were made. Since the variance within observations in one tube was no greater than that between replications, we considered the method employed satisfactory. Examples are presented in Table I.

Table II presents the results from observations of nine replications of eighteen different ketones. An analysis of variance was run on these data; F (cal.) was 1.32, indicating that no significant difference existed between the chemicals, at the 5% level.

4-methyl-3-heptanol is also present in P. barbatus. It was also tested, but because of the low level of response by the ants to this chemical, we concluded that it has no important alarm function.

The results from this experiment might be attributable to either of two possibilities: (1) there is actually no differences in alarm thresholds for the similar chemicals tested or (2) the test was not sensitive enough to detect the differences. The experiment, as already pointed out, has a major drawback in that the actual threshold concentration value cannot be calculated.

Another experiment using the air-stream dilution method of Moser et al. (1968) was undertaken. This technique has the advantages that the actual threshold concentration can be determined and very small amounts of chemi-

TABLE I

REPRODUCIBILITY OF PHEROMONE DIFFUSION COEFFICIENT AT VARYING DISTANCES FROM THE CHEMICAL SOURCE

	Test	Observation	Reaction Time (in 0.5 Seconds)	Distance from source (cm)	$\frac{T}{D^2}$
6-Methyl-5-hepten-2-one:	1	1	90	15.0	.40
	1	2	170	20.0	.42
	2	1	50	13.8	.26
	2	2	105	18.8	.29
	2	3	245	28.8	.29
	2	4	260	30.5	.28
	3	1	105	12.5	.67
	3	2	345	32.5	.32
	4	1	55	12.5	.35
	4	2	85	16.3	.32
	5	1	105	16.3	.40
	5	2	135	17.5	.44
3-Methyl-2-heptanone:	1	1	130	16.0	.50
	1	2	190	32.5	.37
	2	1	45	12.5	.29
	2	2	270	30.5	.29
	3	1	140	17.6	.45
	3	2	240	25.0	.38
	4	1	140	18.8	.40
	4	2	205	22.5	.41

TABLE II

VARIATION IN ALARM ACTIVITY OF ALIPHATIC KETONES WITH *P. BARBATUS*

Chemical	Extremes of Diffusion Coefficient	Mean	Variance
2-Octanone	.56 - .66	.61	.0017
3-Octanone	.46 - .59	.55	.0022
4-Octanone	.44 - .67	.56	.0050
3-Methyl-2-heptanone	.54 - .67	.61	.0023
4-Methyl-2-heptanone	.32 - .69	.56	.0118
5-Methyl-2-heptanone	.44 - .61	.55	.0030
2-Methyl-3-heptanone	.55 - .65	.60	.0023
4-Methyl-3-heptanone	.41 - .64	.56	.0035
5-Methyl-3-heptanone	.55 - .65	.56	.0012
6-Methyl-3-heptanone	.37 - .67	.56	.0070
2-Methyl-4-heptanone	.45 - .63	.55	.0037
3-Methyl-4-heptanone	.46 - .67	.58	.0041
3,4-Dimethyl-2-hexanone	.51 - .70	.58	.0037
2,2-Dimethyl-3-hexanone	.48 - .61	.55	.0022
2,4-Dimethyl-3-hexanone	.55 - .79	.62	.0042
2,5-Dimethyl-3-hexanone	.44 - .64	.55	.0032
2,2,4-Trimethyl-3-pentanone	.54 - .67	.59	.0014
6-Methyl-5-hepten-2-one	.48 - .69	.59	.0045

cals can be used to avoid the danger of overloading the test system.

Table III shows the ketones tested on P. barbatus and the respective K values for each. The total dilution referred to in the table is the syringe dilution plus the cannister dilution.

The difference between K values for two chemicals is probably significant if separated by an order of magnitude. The K values indicate that P. barbatus has a greater sensitivity to 4-methyl-3-heptanone than to most other chemicals. The sensitivity to 4-methyl-3-heptanone was 10,000 times greater than to 3-methyl-2-butanone.

The 4-methyl-3-heptanone K value for P. barbatus as estimated by this procedure is 9×10^{13} molecules/ml of air. For comparison, a few of the ketones were tested on a small colony of P. comanche. These results are given in Table IV. These ants appeared to be able to detect lower concentrations of ketones than P. barbatus. Although the K value of 4-methyl-3-heptanone is the same for both species, P. comanche detected 6-methyl-3-heptanone at 1/100 the concentration required for P. barbatus. These data indicate that there may be variations in K values even among closely related species.

The chromatogram of the steam volatile ether extract of P. barbatus showed several other chemicals besides

TABLE III

EFFECTIVENESS OF DIFFERENT ALIPHATIC KETONES IN ELICITING ALARM BEHAVIOR IN POGONOMYRMEX BARBATUS

Numbers following the names of certain chemicals indicate that no vapor pressure was available for them. For those chemicals the vapor pressure of the footnoted chemicals were substituted.

Chemical	Total dilution	K value
4-Methyl-3-heptanone[1]	$3x10^5$	$9x10^{13}$
6-Methyl-5-hepten-2-one	$3x10^4$	$3x10^{14}$
2,4-Dimethyl-3-hexanone[2]	$3x10^4$	$1x10^{15}$
2-Octanone	$3x10^3$	$3x10^{15}$
3-Octanone[3]	$3x10^3$	$3x10^{15}$
4-Octanone[3]	$3x10^3$	$3x10^{15}$
3-Methyl-4-heptanone[1]	$3x10^3$	$9x10^{15}$
4-Methyl-2-heptanone[1]	$3x10^3$	$9x10^{15}$
4-Heptanone	$3x10^2$	$3x10^{16}$
6-Methyl-3-heptanone	$3x10^2$	$9x10^{16}$
6-Methyl-2-heptanone	$3x10^2$	$9x10^{16}$
2-Methyl-3-heptanone	$3x10^2$	$1x10^{17}$
4-Methyl-3-hexanone[4]	$3x10^3$	$2x10^{17}$
2-Methyl-3-hexanone	$3x10^3$	$2x10^{17}$
5-Nonanone	$3x10^2$	$5x10^{17}$
2-Nonanone[5]	$3x10^2$	$5x10^{17}$
3-Nonanone[5]	$3x10^2$	$5x10^{17}$
4-Nonanone[5]	$3x10^2$	$5x10^{17}$
3-Methyl-2-pentanone[6]	$3x10^2$	$5x10^{17}$
4-Methyl-2-pentanone	$3x10^2$	$5x10^{17}$
2-Methyl-3-pentanone	$3x10^2$	$6x10^{17}$
3-Methyl-2-butanone	$3x10^2$	$2x10^{18}$

[1] 2-methyl-3-heptanone; [2] 2,5-dimethyl-3-hexanone; [3] 2-octanone; [4] 2-methyl-3-hexanone; [5] 5-nonanone; [6] 4-methyl-2-pentanone

TABLE IV

EFFECTIVENESS OF SEVEN ALIPHATIC KETONES IN ELICITING ALARM BEHAVIOR IN POGONOMYRMEX COMANCHE

Chemical	Total dilution	K value
4-Methyl-3-heptanone	$3x10^5$	$9x10^{13}$
2,4-Dimethyl-3-hexanone	$3x10^5$	$1x10^{14}$
3-Octanone	$3x10^4$	$3x10^{14}$
6-Methyl-3-heptanone	$3x10^4$	$9x10^{14}$
2-Octanone	$3x10^3$	$3x10^{15}$
3-Methyl-4-heptanone	$3x10^3$	$9x10^{15}$
2-Methyl-3-heptanone	$3x10^3$	$1x10^{16}$

4-methyl-3-heptanone and its alcohol. The origin of some of these has been traced to the Dufour's gland by dissection and gas chromatography. They are apparently aliphatic hydrocarbons (Wan, 1968).

Discussion

4-methyl-3-heptanone was the first alarm substance to be isolated and identified from ants which was not isoprenoid in nature. The biosynthesis of this compound is unknown at this time.

There may be some phylogenetic significance attached to the presence of 4-methyl-3-heptanone in all Pogonomyrmex species studied but absent in all but one other species of other ant genera studied. Moser et al. (1968) has subsequently reported this compound in Atta texana (Buckley), the Texas leaf cutting ant. There are three possibilities to account for the presence of this chemical in two rather unrelated genera: (1) It might be a remarkable example of convergent evolution; (2) The two genera of ants might be much more closely related than has been thought; or (3) The compound or its direct precursors might be found in the food of both ant genera.

There is no direct evidence for any of the three possibilities. It would seem highly unlikely, however, that of all the thousands of chemicals possible that simple convergent evolution could account for two

unrelated genera having the same alarm substance. This is especially true if the precursors of 4-methyl-3-heptanone are found to be something as simple as acetate, for instance. If the precursors should be much more complex, require only one or two steps to be converted to 4-methyl-3-heptanone, be of such nature that only one isomer was possible, and be present in the food of both Pogonomyrmex ants and Atta texana, then convergent evolution might seem more likely.

The possibility that the two genera of ants are more closely related than has been thought is not difficult to rationalize. Atta texana is a leaf-harvesting, fungus-growing ant. Fungus growing ants are thought to have at first been harvesting ants that gradually evolved and became dependent upon growing fungus for food. The bizarre morphological features and extreme polymorphism might well have evolved quickly after, or as a result of, the evolution of their unusual food habits. Other supporting evidence is: (1) P. badius has polymorphism with large headed soldiers similar to those of A. texana; (2) Both genera of ants use 4-methyl-3-heptanone for the same purpose -- alarm pheromone; and (3) The pheromone is produced by the same gland, the mandibular gland of the head, in both ants. This is constrasted with the fact that the alarm substances of many other ants so far studied are produced by abdominal glands.

Alarm substances so far studied have had two effects on ants. At low pheromone concentration, the effect is attraction. At a higher concentration, an alarm reaction begins in which the ants appear to be extremely aggressive. This system forms a very effective signal. At greater distances from the chemical source, the ants are only attracted toward the source. As they move up the concentration gradient, a concentration is reached that is great enough to release alarm behavior. This threshold concentration is only reached very close to the source. At this point, the ants seem to stop following the chemical gradient and start rapidly moving in concentric circles. With very many ants, this is highly effective means of propagating the pheromone since practically all of the area around the source of the chemical will be investigated very quickly.

The method proposed by Wilson (1963) for the calculation of the alarm chemical threshold, K, was too insensitive to detect the differences in K values of the different chemicals tested. There are two reasons for this: (1) The diffusion coefficient is not readily measurable and can only be estimated, and (2) The smallest amount of chemical that can accurately be injected into the test system is 0.1 micoliters. This size injection apparently overloads the test system. Wilson was able to achieve success because he used ants

as the source of the chemical, thus getting proper sized chemical injections.

With the method of Moser et al. (1958), the K values for several ketones were determined. P. barbatus and P. comanche are more sensitive to 4-methyl-3-heptanone than to other closely related ketones. There is apparently no simple correlation between chemical structure and ability to initiate a behavioral response in the ants. However, it appears that only chemicals with a molecular weight similar to 4-methyl-3-heptanone produce alarm behavior at very low threshold concentrations.

The 4-methyl-3-heptanone K value of P. barbatus as estimated by this procedure is 9×10^{13} molecules/ml of air. This agrees well with the value of 4.47×10^{13} molecules/ml calculated for P. badius by a different method (Bossert and Wilson, 1963). Pogonomyrmex ants are apparently much less sensitive to 4-methyl-3-heptanone than Atta texana. Moser et al. (1968) reports a K value of 2.66×10^{7} molecules/ml for A. texana estimated by the same technique used in this study.

Possibly, Atta ants rely on olfaction to a greater extent than P. barbatus. Atta ants will form odor trails to food only a few cm from the nest opening, while P. barbatus does not establish trails to food sources at distances of up to two meters in the laboratory. Even under natural conditions where Pogonomyrmex ants commonly

build trails of fifty or more meters, the physical outline of the trail may be as important as any odor associated with it. It is possible that the olfactory sense organs are not as highly developed in P. barbatus as in A. texana.

Cavil et al. (1967) has reported the presence of aliphatic hydrocarbons from the Dufour's gland of Myrmecia gulosa (F.). Specifically identified were cis-heptadec-8-ene (62 per cent), pentadecane (17 per cent), and heptadecane (4 per cent). The function in these ants was suggested to be to lubricate the sting. The hydrocarbons from the Dufour's gland of P. barbatus are apparently all saturated. Their function is unknown.

CHAPTER III

IDENTIFICATION OF HYDROCARBONS FROM NOVOMESSOR COCKERELLI

Hydrocarbons have been known for some time to occur in the exoskeleton and occasionally the hemolymph of insects (Dennell and Malek, 1956; Baker et al., 1960; Gilby, 1962; and Acree et al., 1965). They have most often been associated with the waxy cuticle and have been in the C-25 to C-45 range.

Cavill and Williams (1967) have reported the presence of aliphatic hydrocarbons as products of the Dufour's gland in the ant Myrmecia gulosa (F.). Likewise, Bernardi et al. (1967) have reported the presence of n-undecane, n-tridecane and n-pentadecane in Lasius (Dendrolasius) fuliginosus Latr. gasters.

This paper reports on the presence of several hydrocarbons in the gaster of Novomessor cockerelli (E. André).

Experimental Methods and Results

The gasters were cut from 10,000 (35 gm.) N. cockerelli collected near Tucson, Arizona, in the fall of 1966. The gasters were homogenized in distilled water in a Waring blender and steam distilled until about 1500 ml of distillate

was collected. This was extracted with ether using a continuous vapor-vapor extractor and concentrated to about 2 ml by distillation through a spiral column. This extract showed twelve major peaks when analyzed by gas chromatography (.02 in. x 450 ft, interior coated with polymetaphenyl ether (6 rings), 145° C, 35 psi helium, Figure 2).

An infrared spectrum of the crude extract showed strong C-H stretching at 2962 cm^{-1} and 2872 cm^{-1} along with C-H bending vibrations at 1375 cm^{-1} and 1450 cm^{-1}. There was no absorption in the regions for unsaturation or carboxyl groups, indicating that the major portion of the extract consisted of saturated hydrocarbons.

The extract was analysed on a combination gas chromatograph-mass spectrometer (Ryhage et al., 1965). The mass spectra for the unknowns and standards are presented in Figures 3 through 18. Aliphatic saturated hydrocarbons are easily identified by their mass spectra (Budzikiewicz et al., 1964). They are characterized by a gradual decrease in relative intensity of the produced fragmentation ions as the ions get larger. Molecules which are branched or have unsaturation have preferred sites of fragmentation, resulting in a disruption of the gradual decrease in relative intensity for larger ions. Frequently the parent ion also is much reduced or absent in branched hydrocarbons.

From the mass spectral data, the major peaks of the extract were identified as follows: peak 2, a branched isomer of decane; peak 3, n-tridecane; peak 4, n-tetradecane;

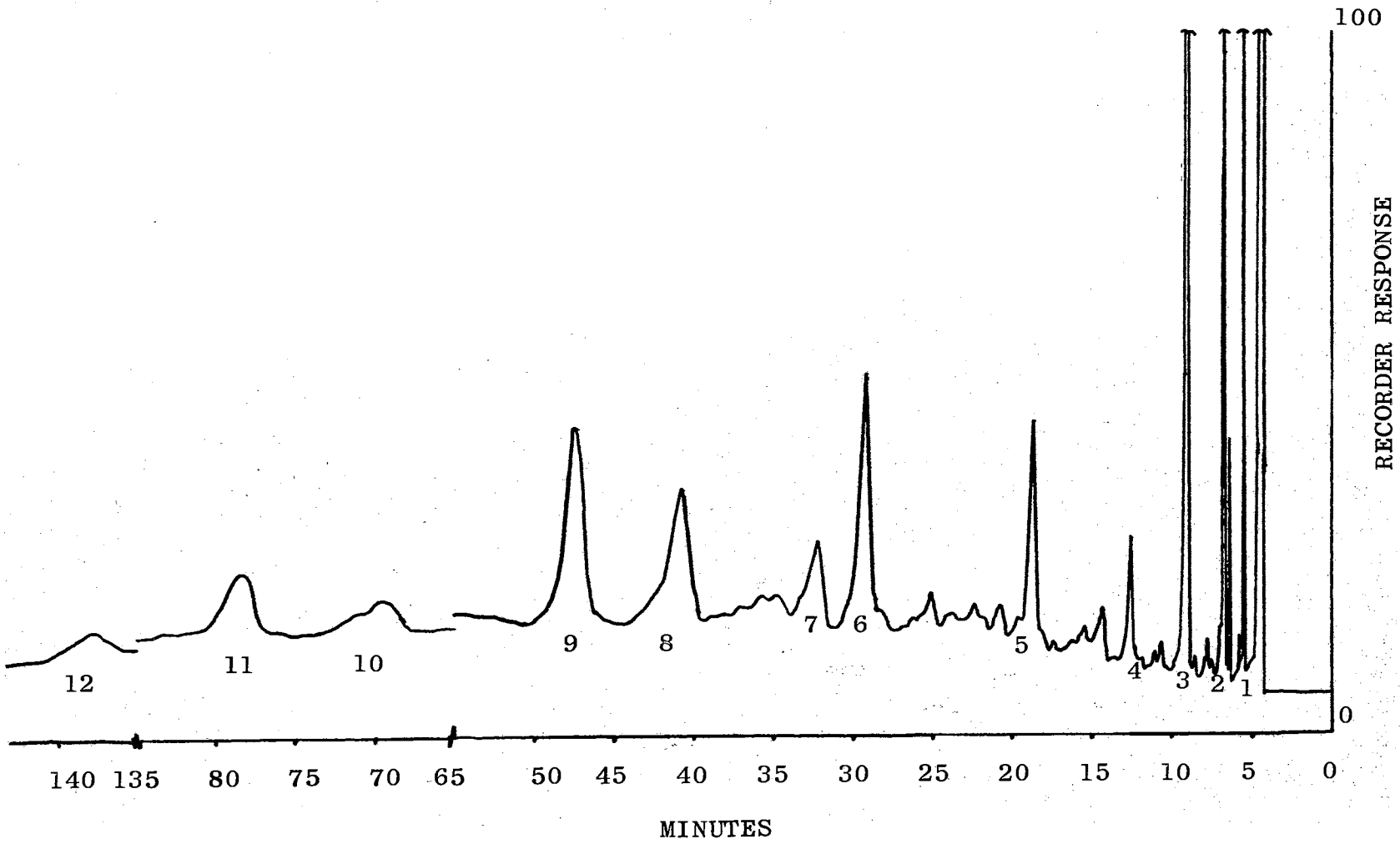

Figure 2. Gas Chromatogram of Steam Volatile Chemicals from N. Cockerelli

peak 5, n-pentadecane; peak 6, n-hexadecane; peak 9, n-heptadecane; peak 11, n-octadecane and peak 12, n-nonadecane. The identity of peaks 3,4,5,6,9,11, and 12 were confirmed as having the identity given above by comparison of mass spectra and gas chromatograph retention times of known standards to those of the chemicals in the extract.

Peak numbers 1,2,7,8, and 10 were not identified. Peak number 2 appears to be a branched isomer of decane. Peak number 10, likewise, seems to be a branched isomer of octadecane. Their spectra are given in Figures 4 and 15, respectively. The fact that the retention time of peak 10 is less than for peak 11, n-octadecane, helps substantiate its proposed identity. Satisfactory mass spectra were not obtainable for peaks 7 and 8. It was concluded that their parent ions were not observed. Peak 1, the mass spectrum of which is shown in Figure 3, was unidentified.

Although an extract from the heads and thoraxs was prepared in the same way as the abdomens, none of the hydrocarbons observed in the abdomen were found.

Even though N. cockerelli ants do not have stings, the poison sac and poison gland and the Dufour's gland or accessory gland are retained as in stinging ants. In addition, there are several glands or reservoirs, to be called the anal glands, opening dorsally near or into the cloacal opening. Each of these is composed of a few

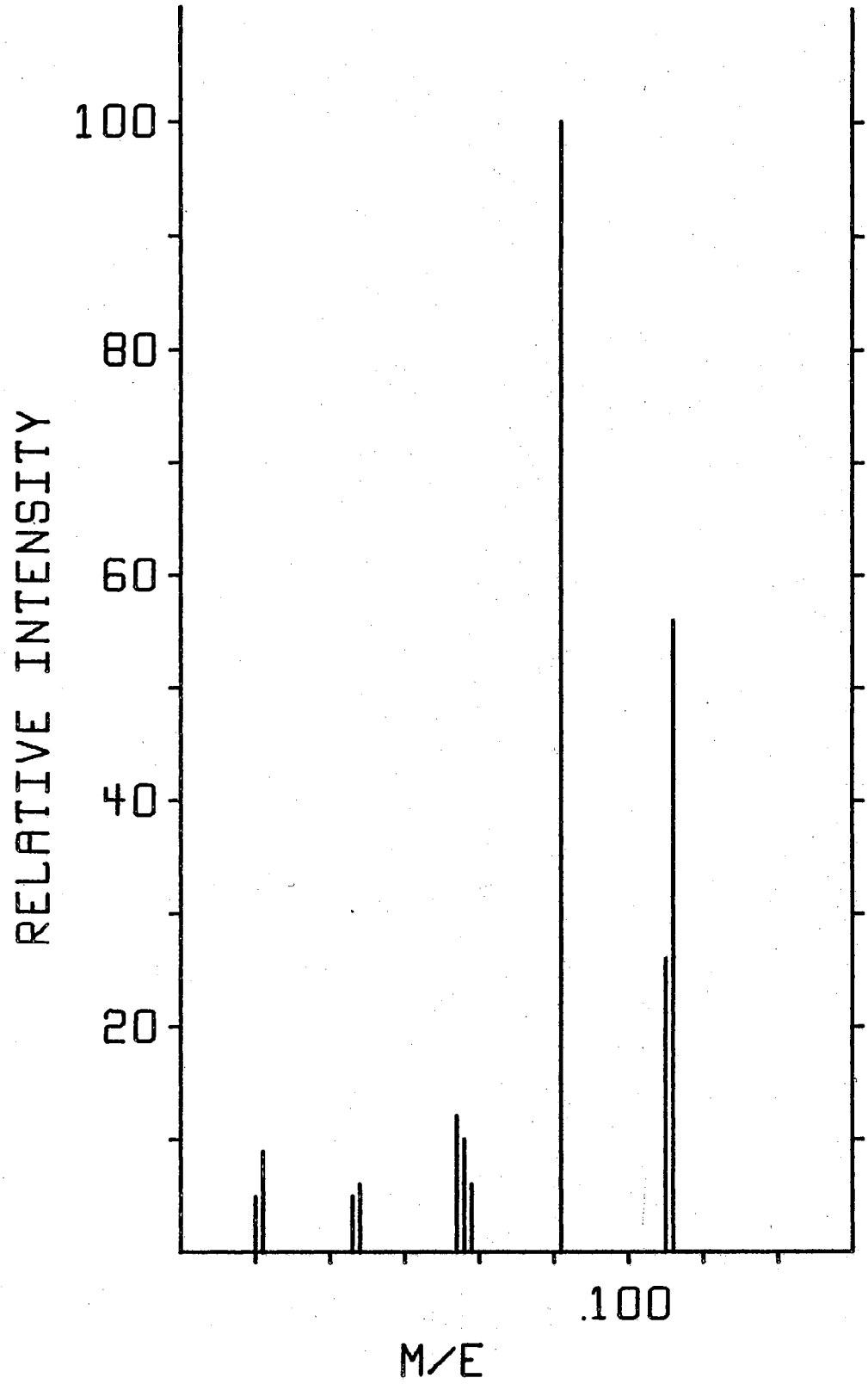

Figure 3. Mass Spectrum of Unknown From N. cockerelli

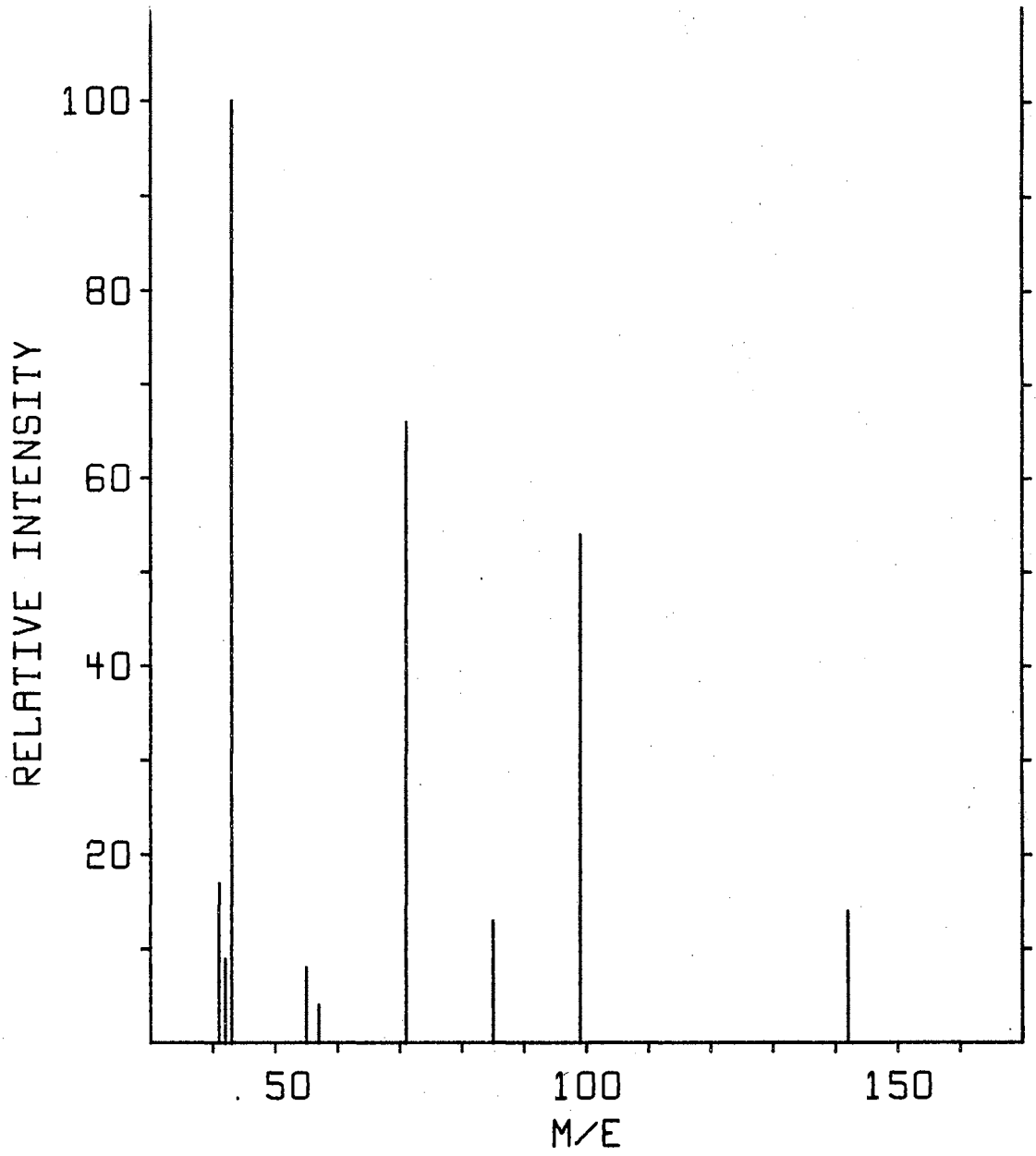

Figure 4. Mass Spectrum of Unknown from N. Cockerelli

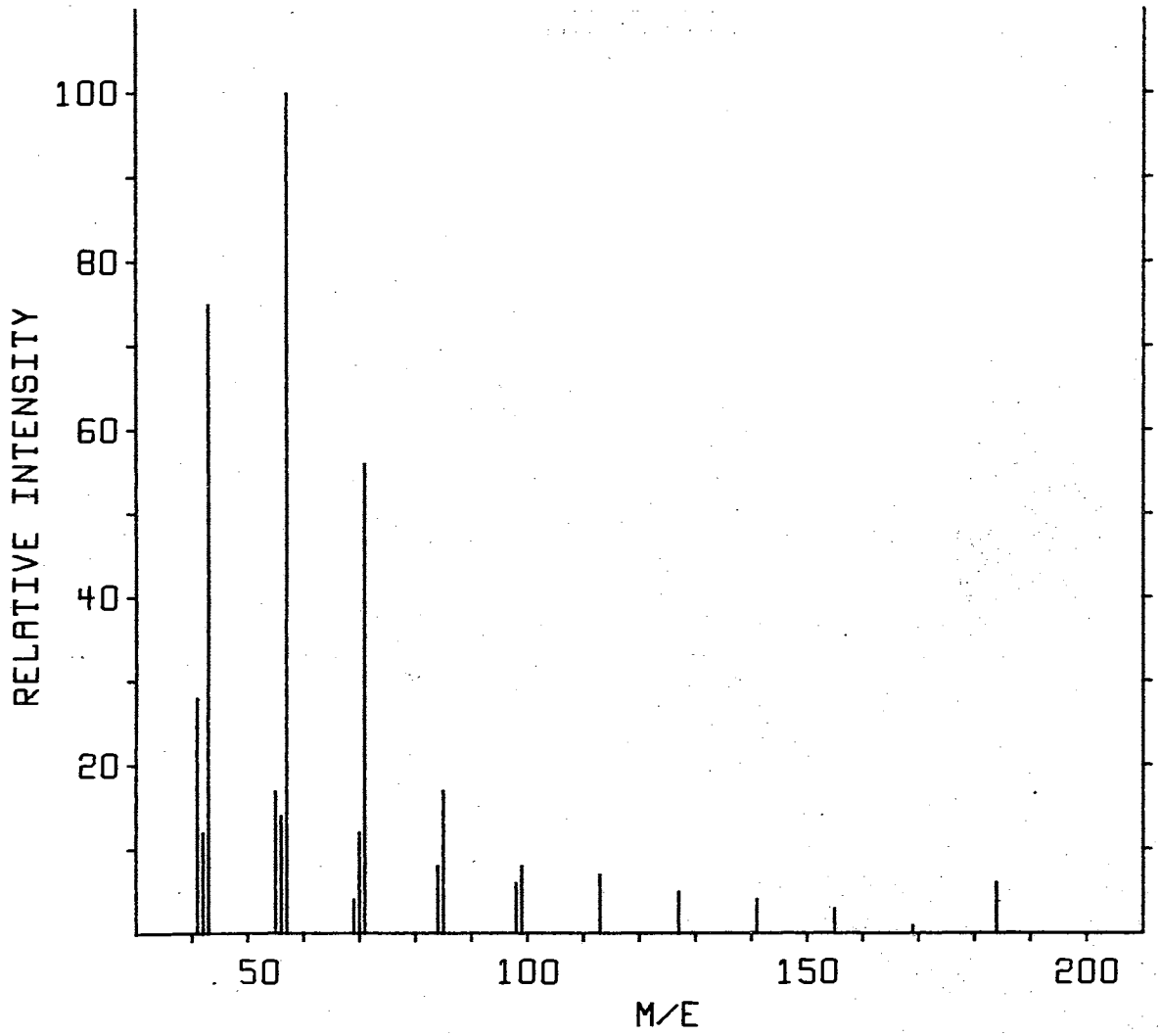

Figure 5. Mass Spectrum of N-tridecane identified from N. Cockerelli

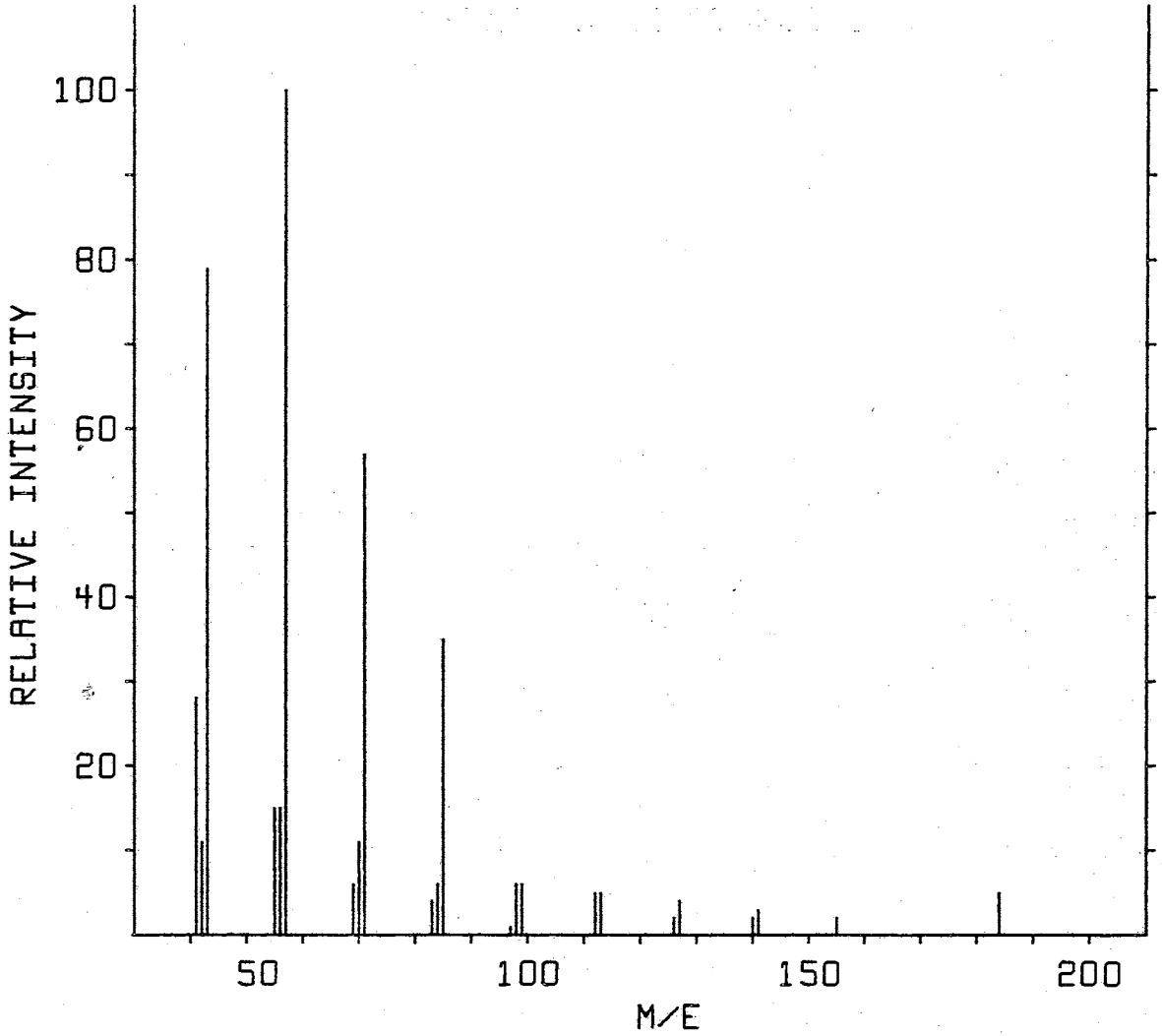

Figure 6. Mass Spectrum of
Authentic N-tridecane

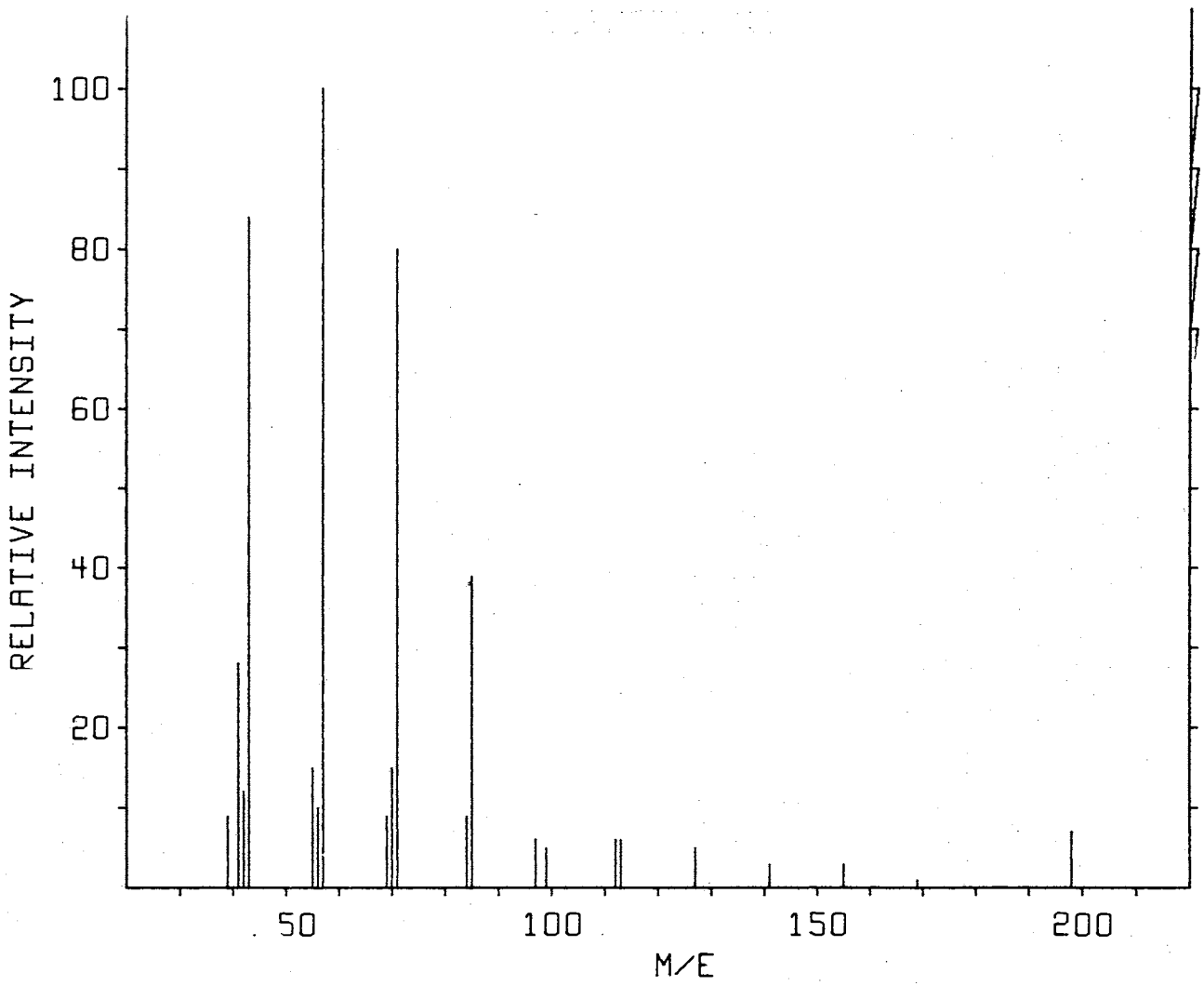

Figure 7. Mass Spectrum of N-tetradecane Identified from N. Cockerelli

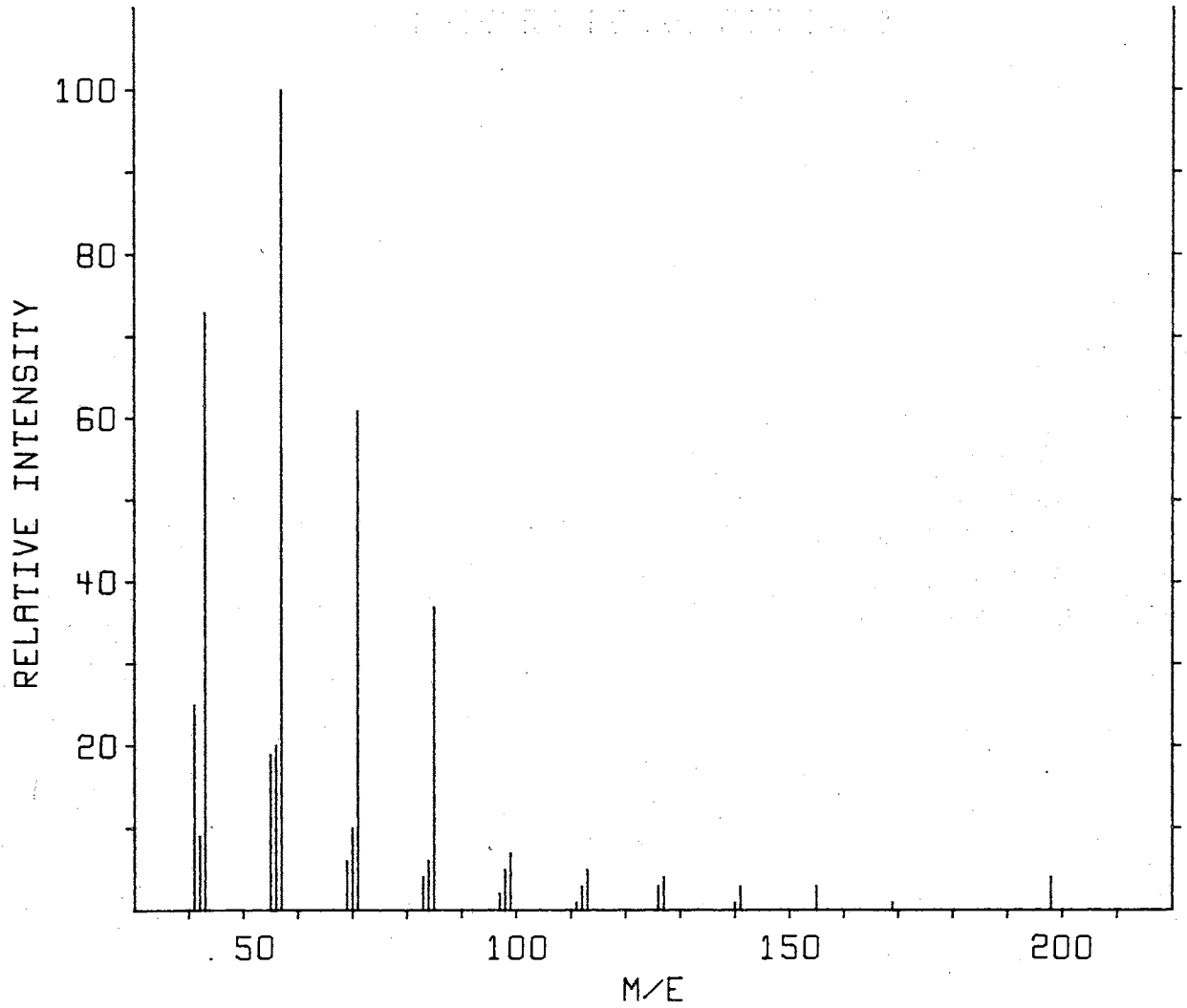

Figure 8. Mass Spectrum of Authentic N-tetradecane

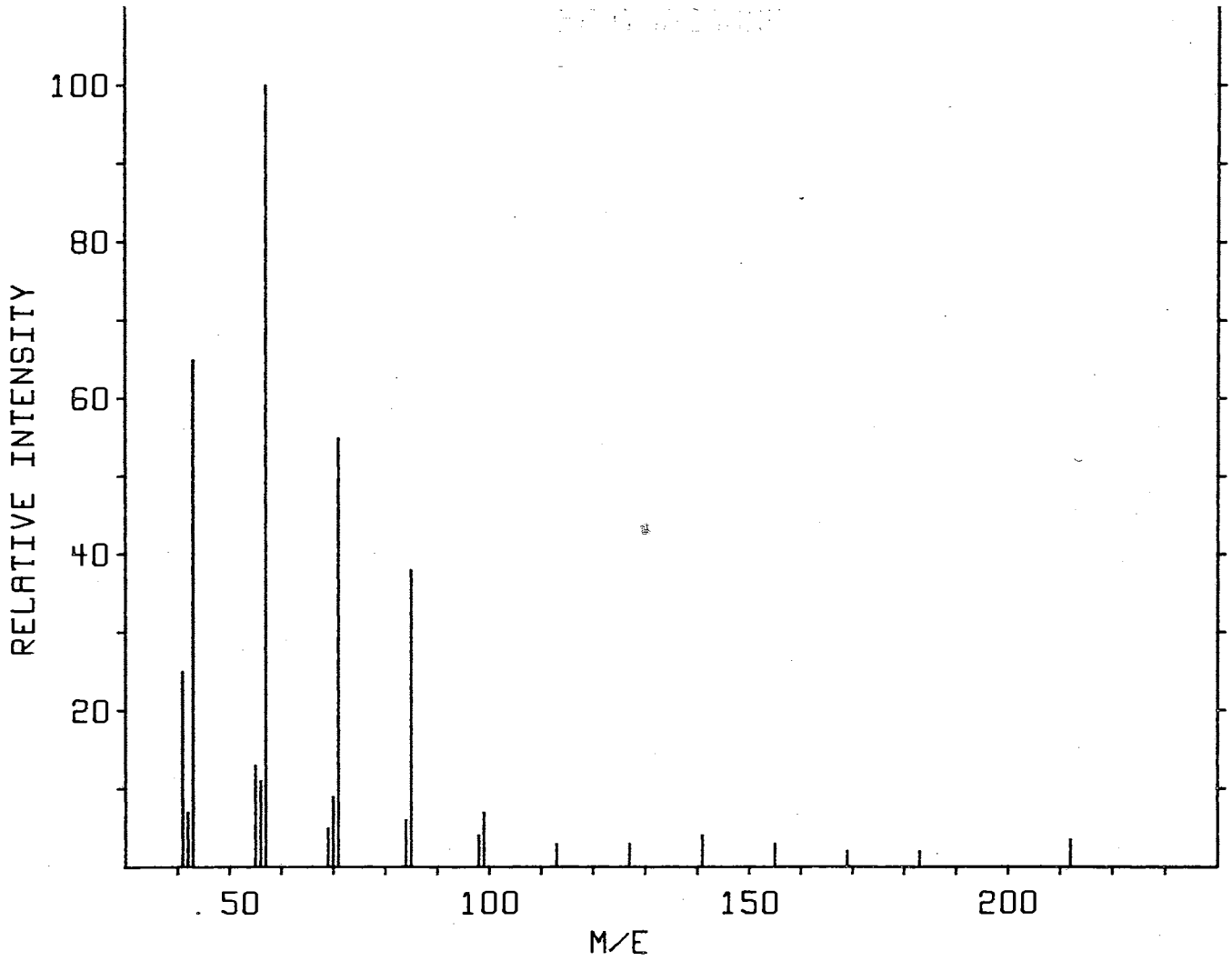

Figure 9. Mass Spectrum of N-pentadecane Identified from N. Cockerelli

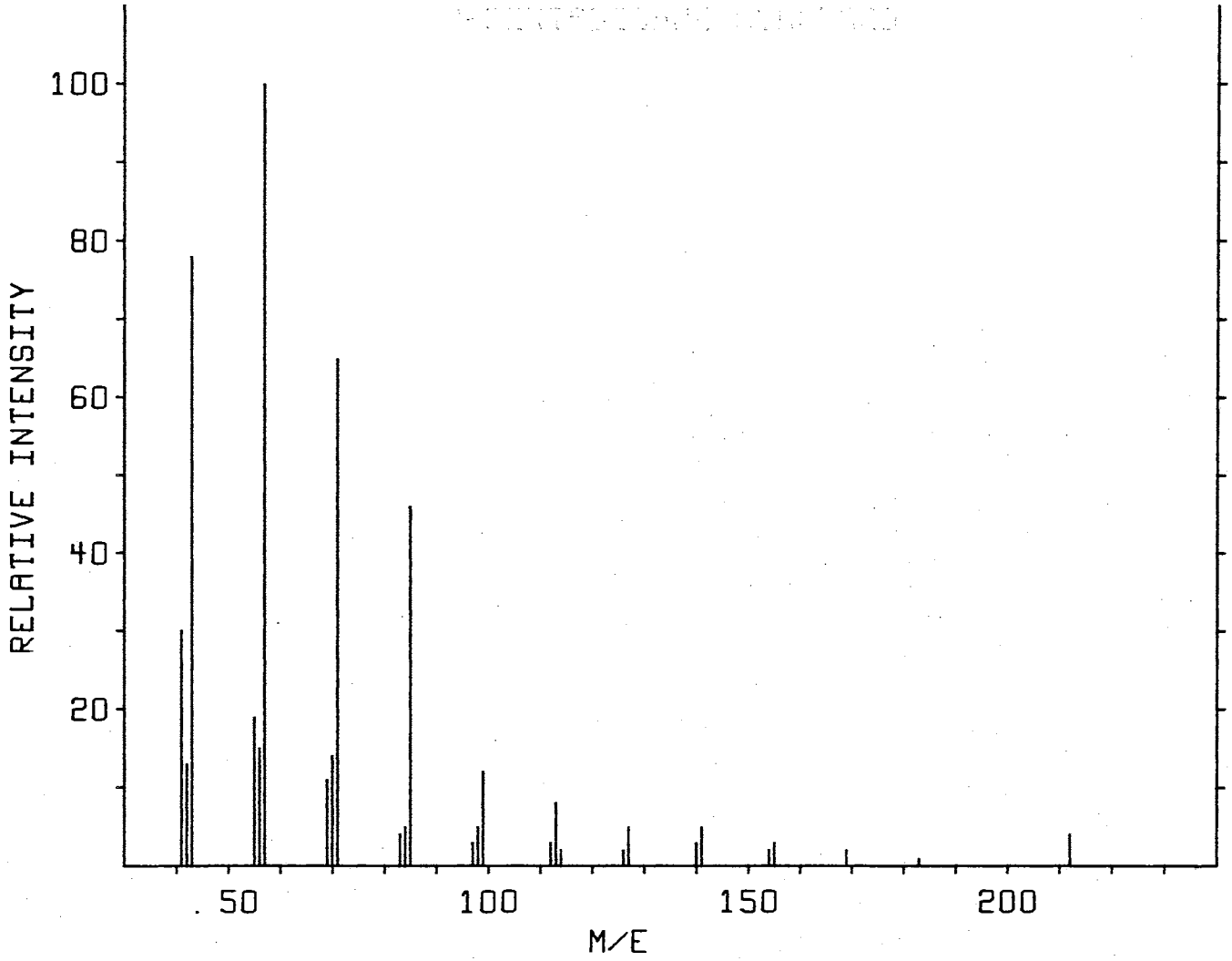

Figure 10. Mass Spectrum of
Authentic N-pentadecane

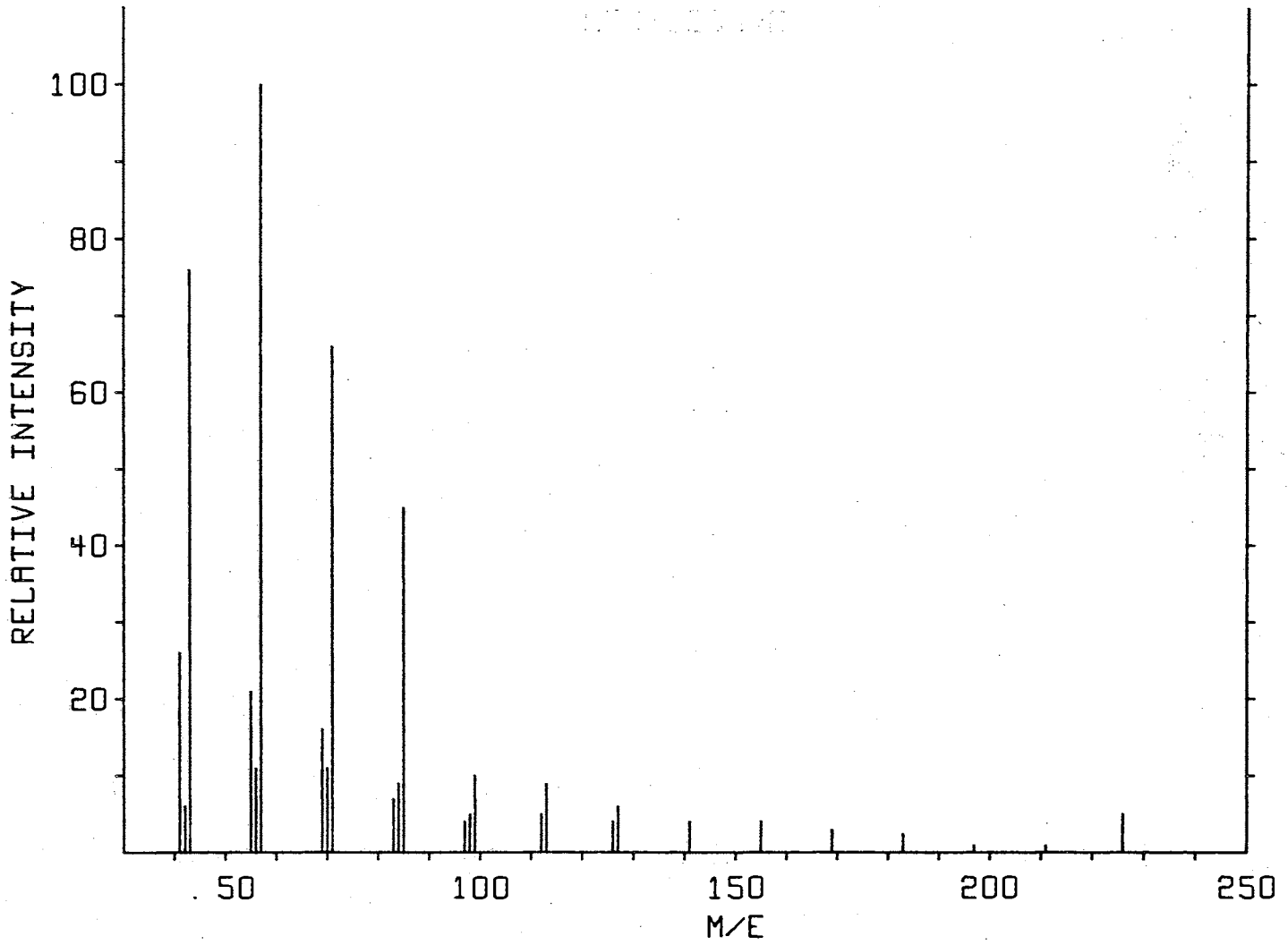

Figure 11. Mass Spectrum of N-hexadecane Identified from N. Cockerelli

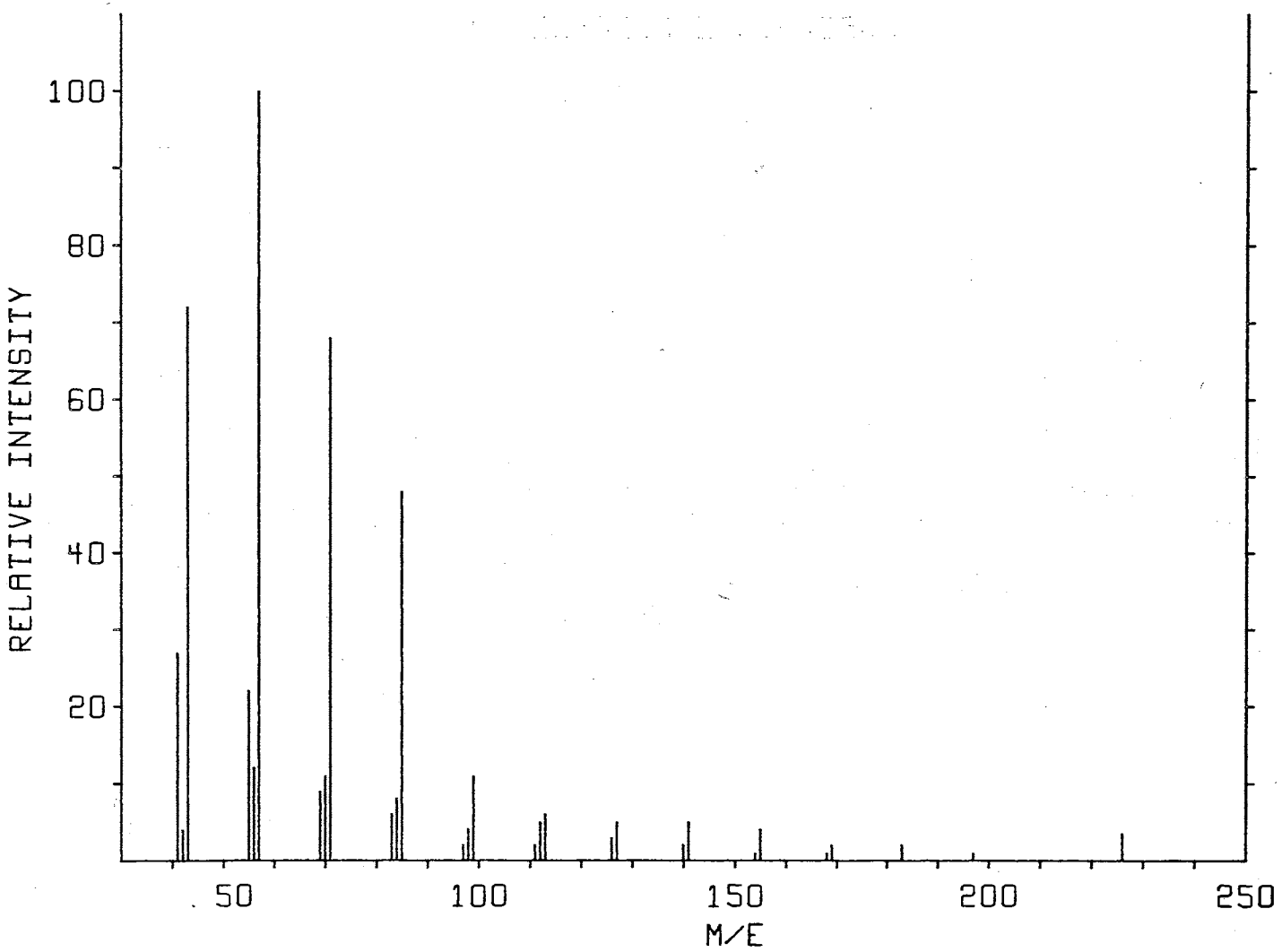

Figure 12. Mass Spectrum of Authentic N-hexadecane

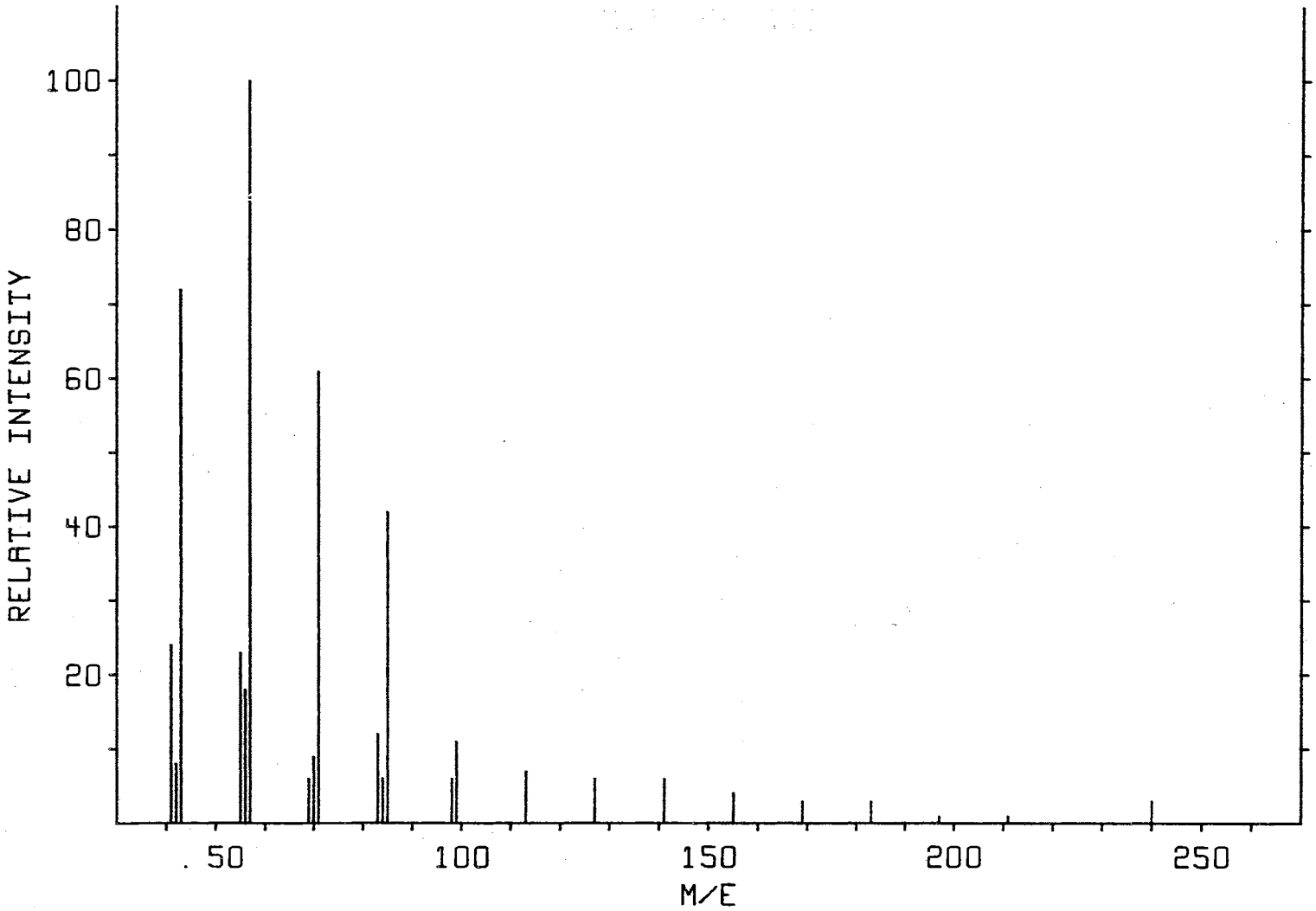

Figure 13. Mass Spectrum of N-heptadecane
Identified from N. Cockerelli

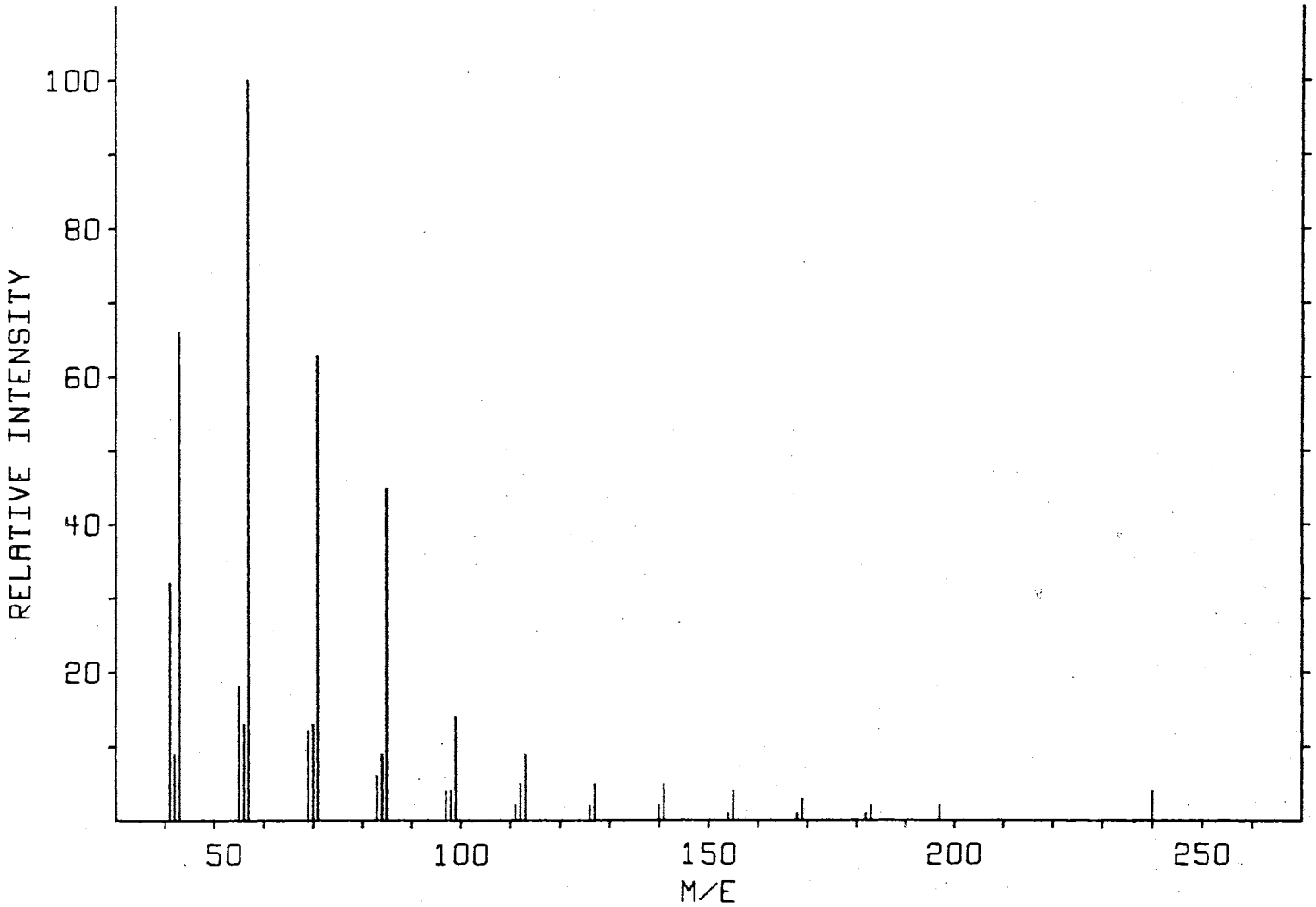

Figure 14. Mass Spectrum of Authentic N-heptadecane

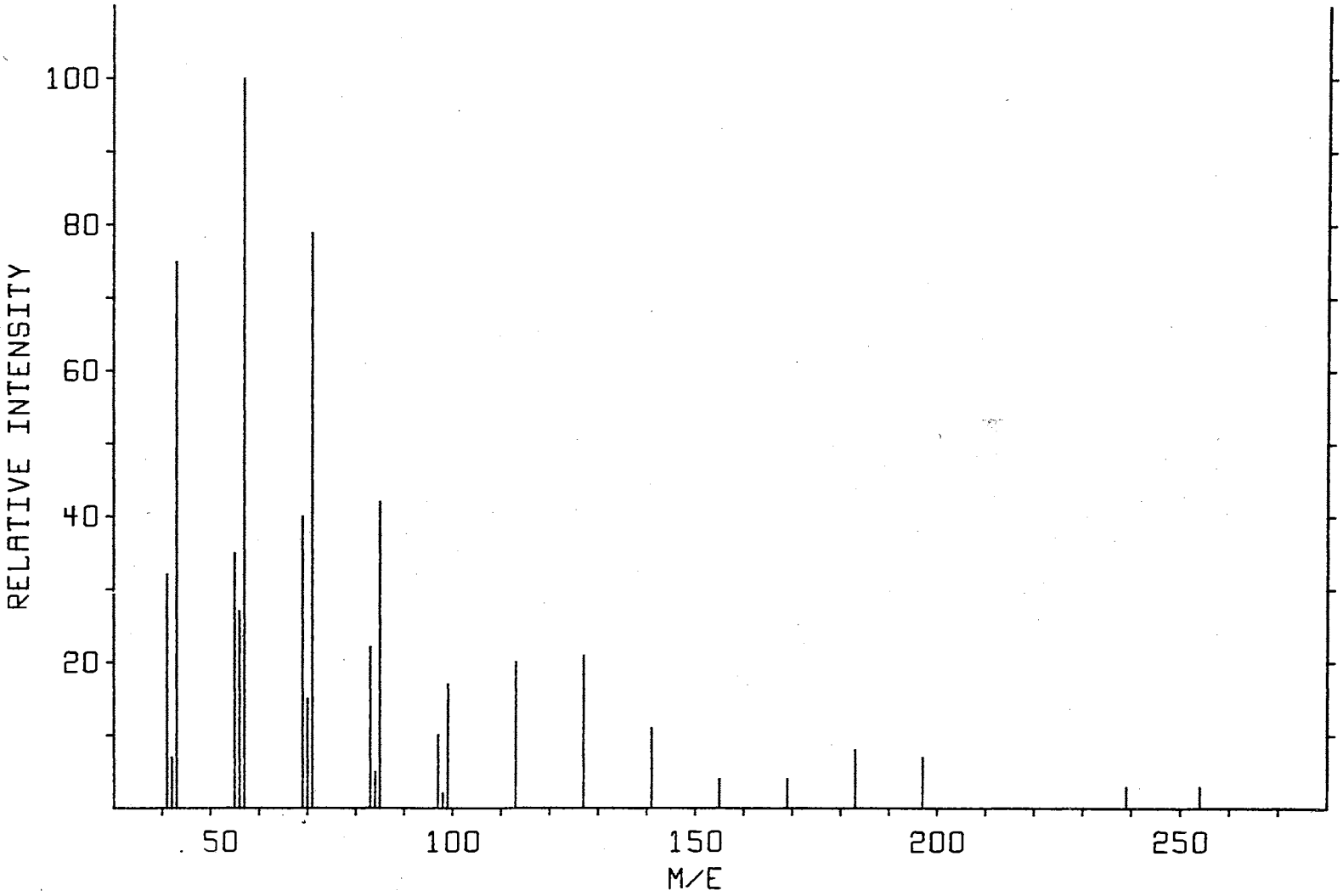

Figure 15. Mass Spectrum of Unknown from N. Cockerelli

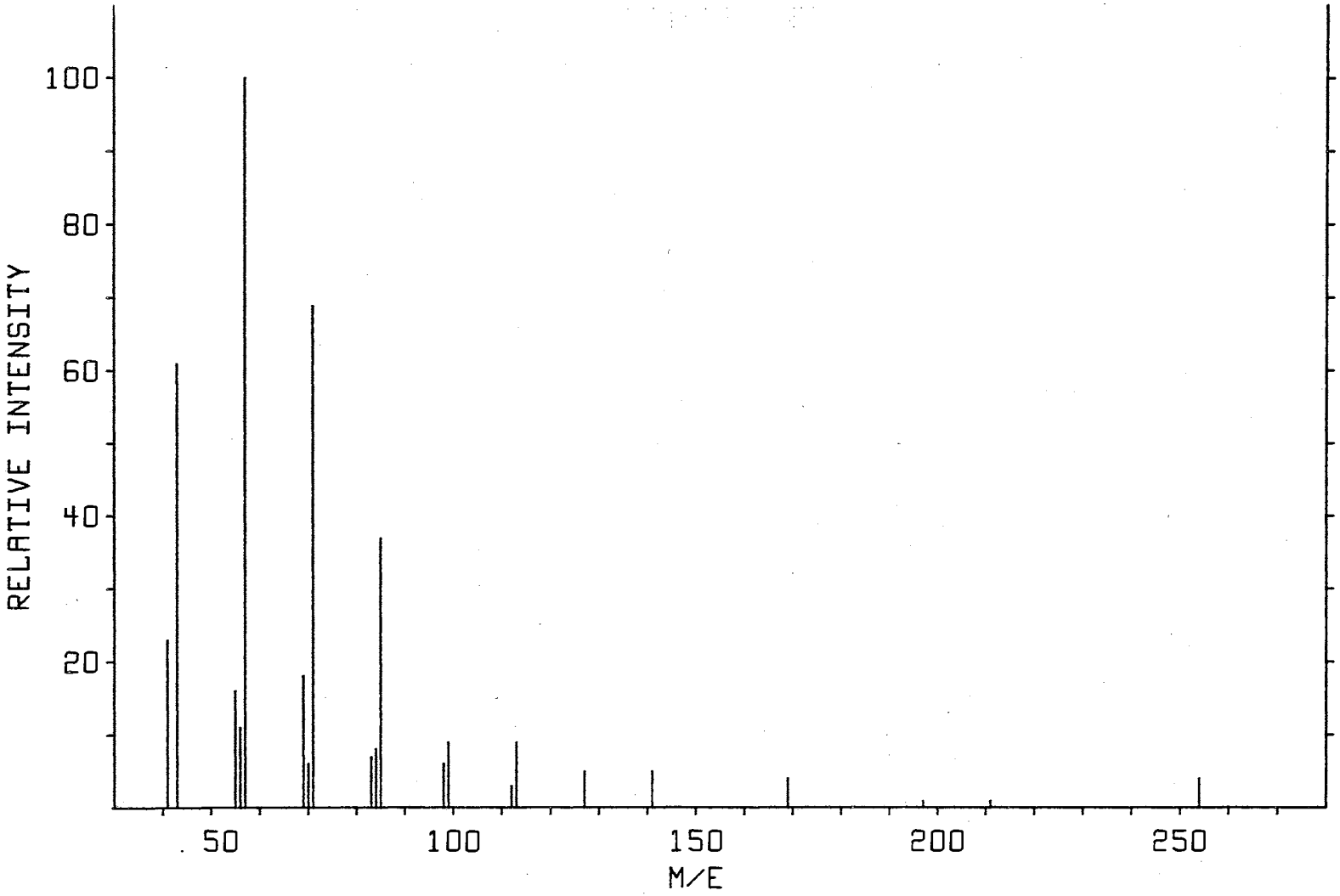

Figure 16. Mass Spectrum of N-octadecane from N. Cockerelli

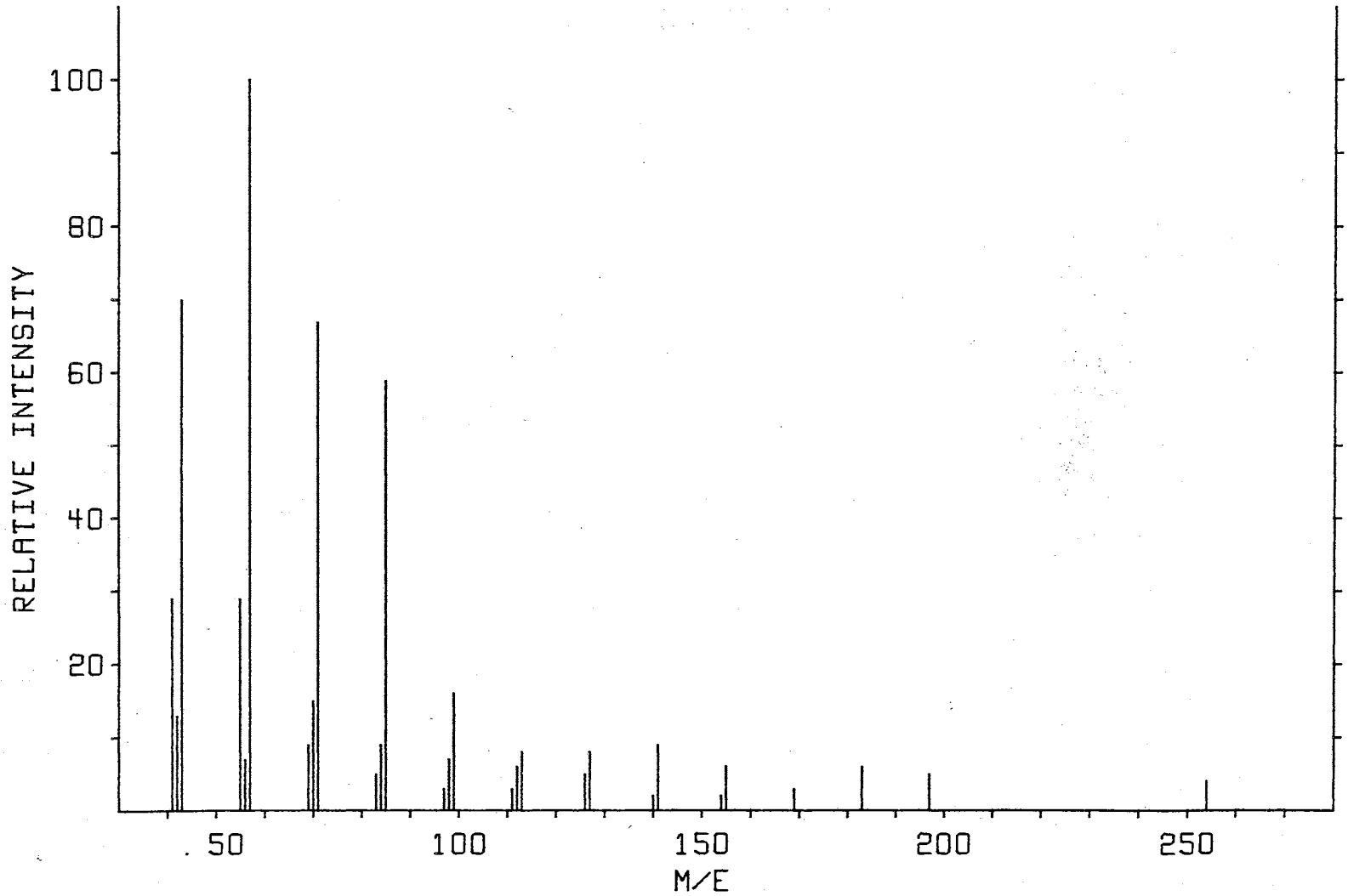

Figure 17. Mass Spectrum of Authentic N-octadecane

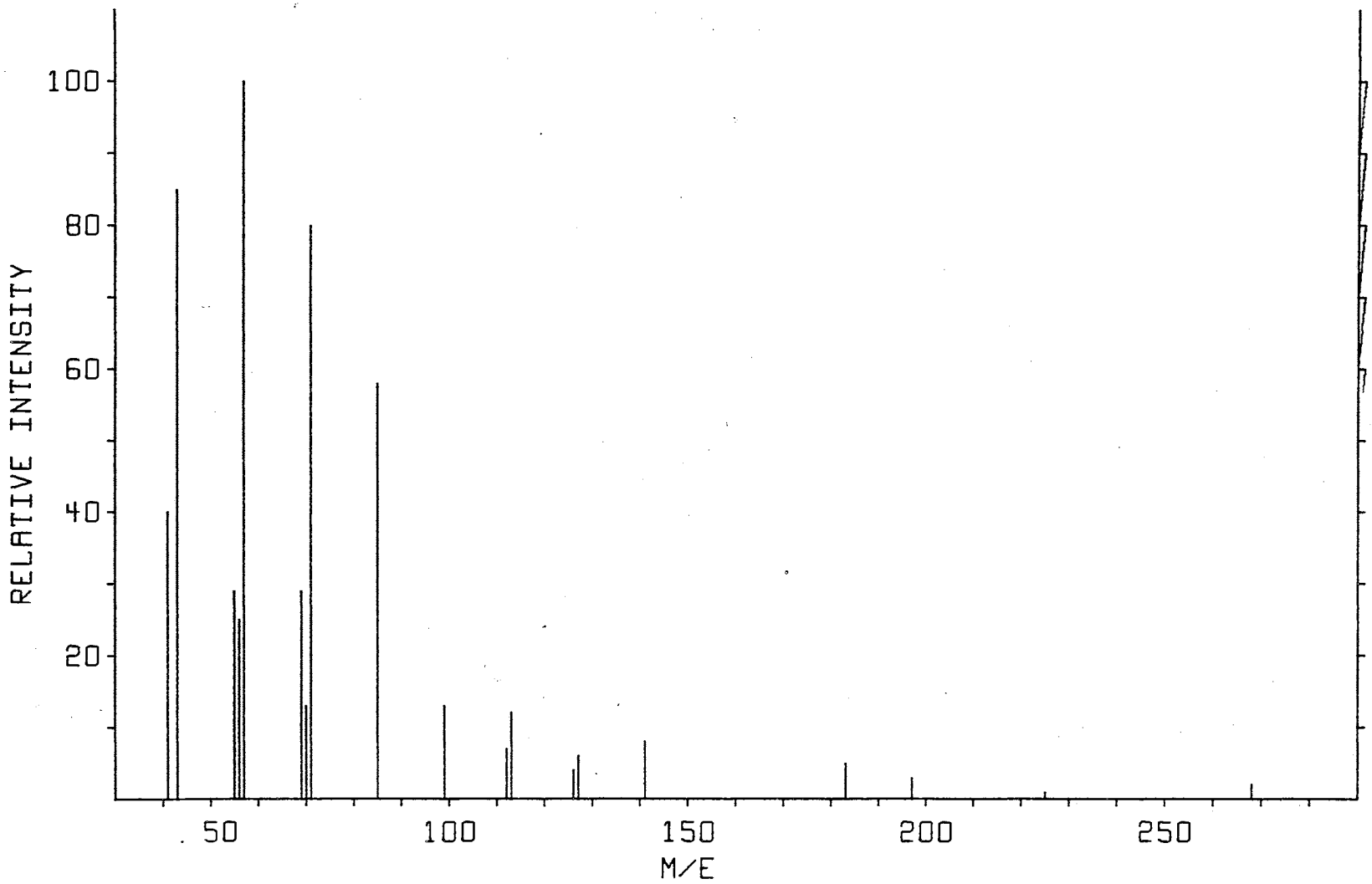

Figure 18. Mass Spectrum of N-nonadecane Identified from N. Cockerelli

linearly arranged globules becoming progressively smaller distally from their insertion at the anus. No other glandular tissue can be seen attached to them and they seem to float free in the hemolymph distally. They are filled with a light yellow liquid similar to that seen in the poison sac and in the glands to be described next.

A very large gland, the dorsal gland, occupies the dorsal part of the gaster above the digestive tract. It varies in size, but often it extends from the posterior to the anterior end of the gaster and from side to side laterally. It is composed of countless small round yellow liquid filled globules apparently connected together by what may be glandular tissue. When these globules are broken under the water in which the ants are dissected, an oil slick forms on the surface of the water. No connection could be found between this dorsal gland and the outside of the body, the digestive tract or any of the other three glands previously mentioned.

Because of the small quantities of hydrocarbons involved, only the origin of the major component, tridecane, was determined. This was done by gas chromatography both by heating the individual glands in a pyrolysis loop and by making ether extracts of the glands. Tridecane was found in greatest concentrations in the dorsal gland, but it was also found in the posterior portion of the digestive

tract, the anal glands and the poison sac and Dufour's gland. Tridecane was not found in the exoskeleton.

Discussion

Eight hydrocarbons have been characterized from the gasters of Novomessor cockerelli. These are a branched isomer of decane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, and nonadecane.

The exact origin of these hydrocarbons is uncertain. It seems possible that the hydrocarbons are produced by the dorsal gland and then released into the haemolymph to be picked up by the anal gland, the Malpigian tubules, the Dufour's gland, and the poison sac. This is suggested by the quantities of tridecane found in the four glands.

The function of the hydrocarbons is not known. The function suggested by Cavill and Williams (1967) for the hydrocarbons found in the Dufour's gland of Myrmecia gulosa is that of lubricating the sting. However, N. cockerelli has no sting so that function could not apply. Also, the presence of the hydrocarbons in glands having no evolutionary connection with the sting apparatus would suggest another function. N. cockerelli have a repugnant smelling oily chemical which they squirt from the anal area when frightened. Although none of the hydrocarbons identified have a smell similar to the smell of the defensive chemical, it is very possible that the hydrocarbons might serve as

a solvent and a cuticle penetrating agent for this defensive chemical.

It is interesting to note that the hydrocarbons found by Cavill and Williams (1967) and Bernardi et al. (1967) all have odd number carbon chain length. Although, the biosynthesis of aliphatic hydrocarbons is not known, their similarity to fatty acids certainly was recognized. With the presence of both odd and even numbered carbon chain lengths, the relationship of these hydrocarbons to the fatty acids may be in doubt.

CHAPTER IV

THE VALUE OF VOLATILE CHEMICALS FROM ANTS AS A TAXONOMIC TOOL

Gas chromatographic data has been used for some time in plant taxonomy (Alston, 1963), where the distribution of secondary plant metabolites has been shown to have some phylogenetic significance. This technique has not been used in insect taxonomy.

Law et al. (1965) suggested that the volatile chemicals released by male ants might have taxonomic importance. Indeed, 4-methyl-3-heptanone has been shown to be present in all Pogonomyrmex species studied (McGurk et al., 1966), but this chemical has been found in only one other genera (Moser et al., 1968). Also iridodial, a methylcyclopentane monoterpene, seems to be widely distributed in the subfamily Dolichoderine, but has been found in no other subfamily of ants (McGurk et al., 1968).

This study is an attempt to determine the usefulness of gas chromatography for ant taxonomy.

Experimental Methods

Freshly collected ant species used in this study were: Pogonomyrmex barbatus, Forelius foetida (Buckley), Dorymyrmex pyramicus (Roger), Iridomyrmex pruinosus analis (E. Andre), Tapinoma sessile (Say), Trachymyrmex septentrionalis obscura (Wheeler), and Pheiodole dentata Mayr. Acromyrmex (Moellerius) versicolor (Pergande) and Novomessor cockerelli had been collected about ten months previously and kept frozen at -18° until used.

The chromatograms presented in Figures 19-26 were all obtained under as nearly identical conditions as possible over a span of two days. The column and conditions used for those chromatograms were as follows: 0.02 in. x 450 ft capillary column lined with a film of Monsanto OS-138 (a six-ring polyphenyl ether, bis m- (m - phenoxyphenoxy) phenyl ether) at 33 psi helium, 145° C. The ants to be chromatographed were placed in the closed pyrolysis loop of an Instruments, Inc. model 393 gas chromatograph and heated to 200° C. The vapors were then diverted into the column described above. An internal standard, 2-methyl-3-octanol, was injected into the closed pyrolysis loop before the diversion of the ant vapors into the column. The internal standard was thus co-chromatographed with the volatile chemicals from the ants.

The iridodial isomer variation was determined for the species Tapinoma sessile, Iridomyrmex pruinosus, and Dory-

myrmex pyrimicus. This was done by reducing the iridodials in the ether extract from 200 ants with excess lithium aluminum hydride. The resulting alcohols were then converted to silyl ethers as reported by McGurk et al. (1968). Standards were prepared from nepetalinic acids with known isomeric configurations.

Results

The gas chromatograms of the ant volatile chemicals are presented in Figures 19 to 26 for the species studied. The largest, most significant peaks have been numbered on each of the chromatograms. The chromatograms are not drawn to the same scale.

To correct for retention time variations, the retention time of the internal standard co-chromatographed with each analysis was divided into the retention time for each numbered chromatographic peak. These ratios are presented in Table V. The peaks resulting from the internal standards, of course, have a ratio of 1.00. The ratios presented are the averages of 2 to 6 analyses for each species.

To measure the variability of ratios presented in Table V, six analyses were run on F. foetida. These data are presented as internal standard ratios in Table VI. The standard deviation is also shown for each peak ratio. Statistically, it is to be expected that approximately 95% of the time a ratio which falls outside of the interval

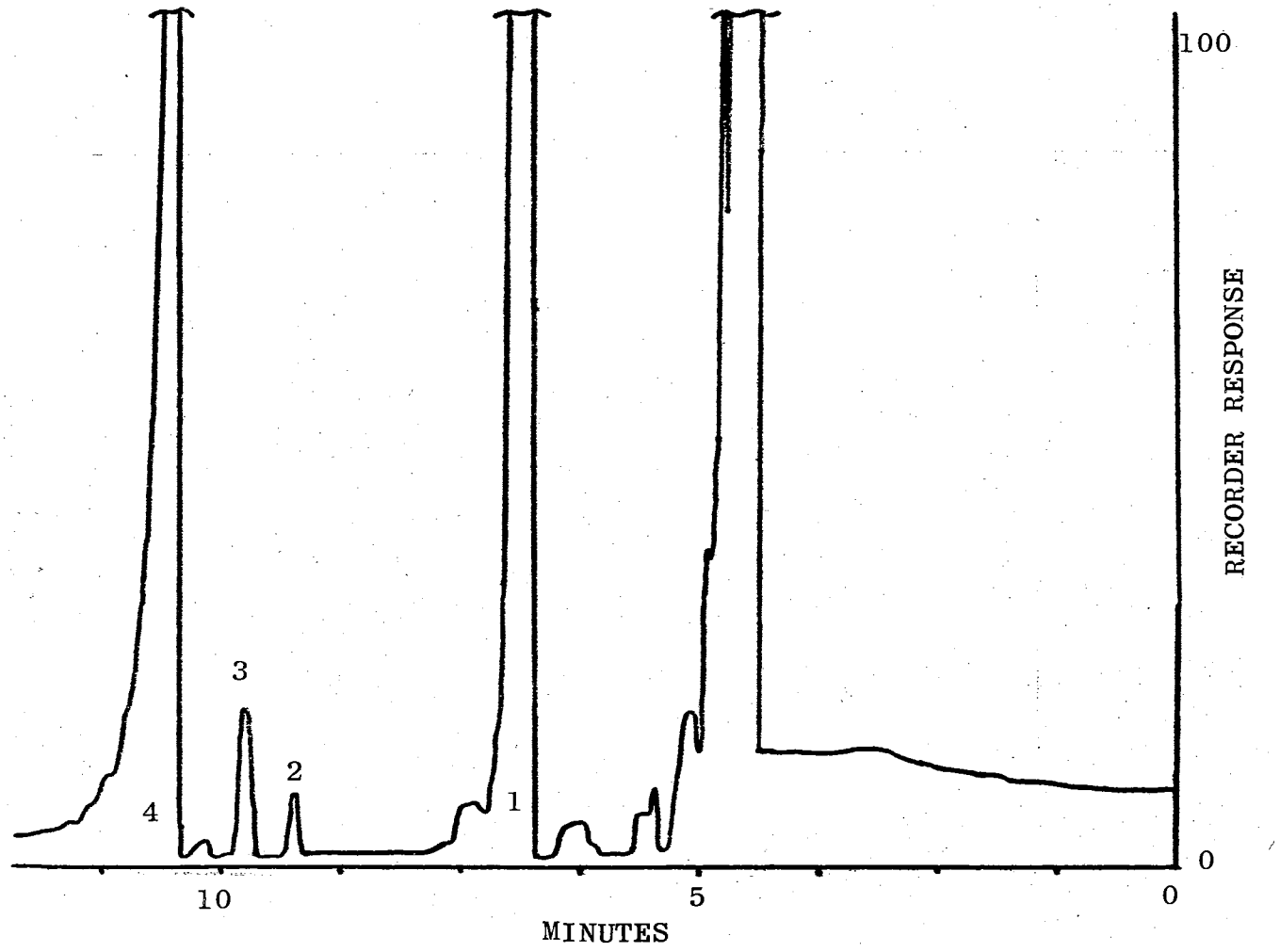

Figure 19. Gas Chromatogram of Volatile Chemicals from Forelius foetida

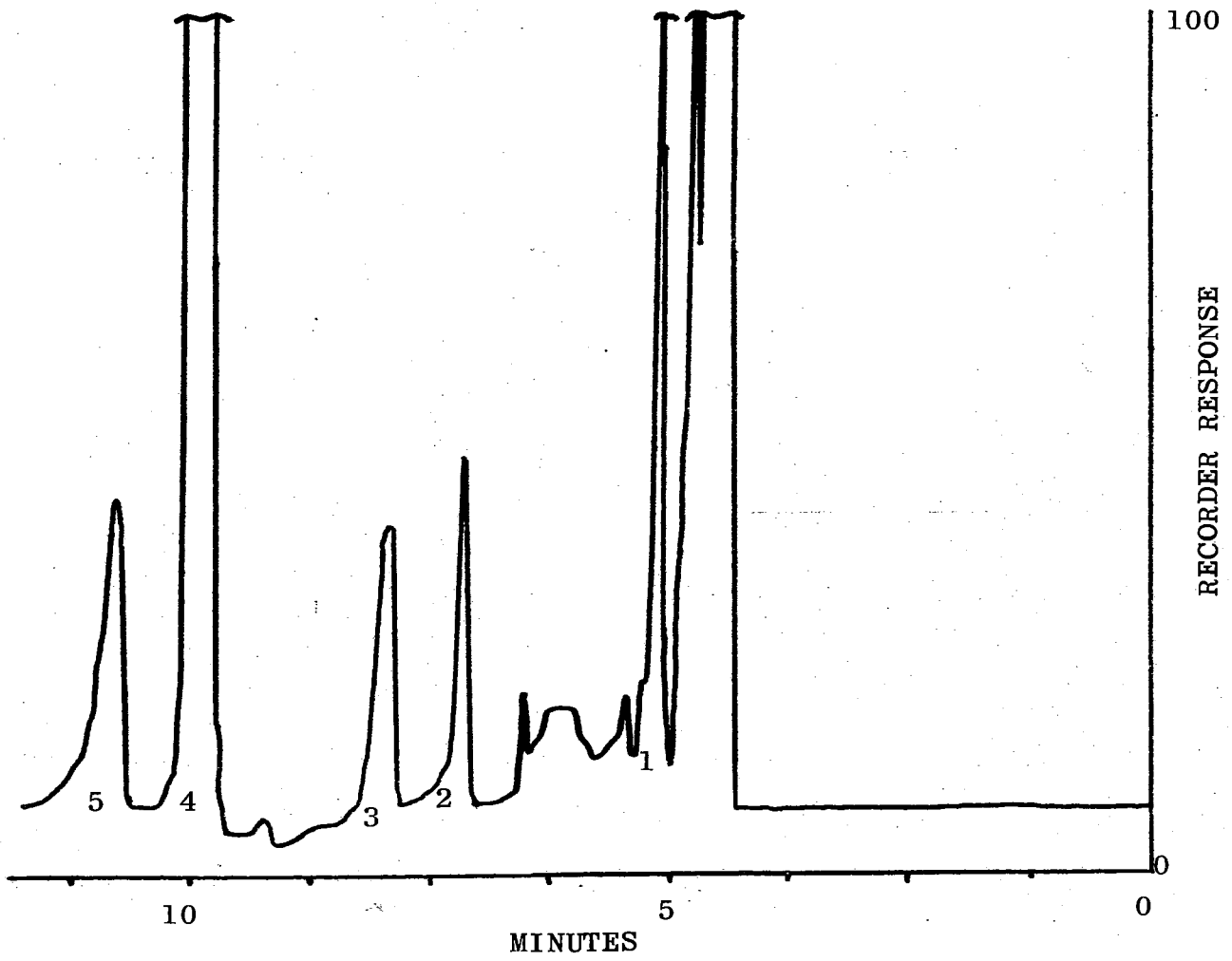

Figure 20. Gas Chromatogram of Volatile Chemicals from Dorymyrmex pyramicus

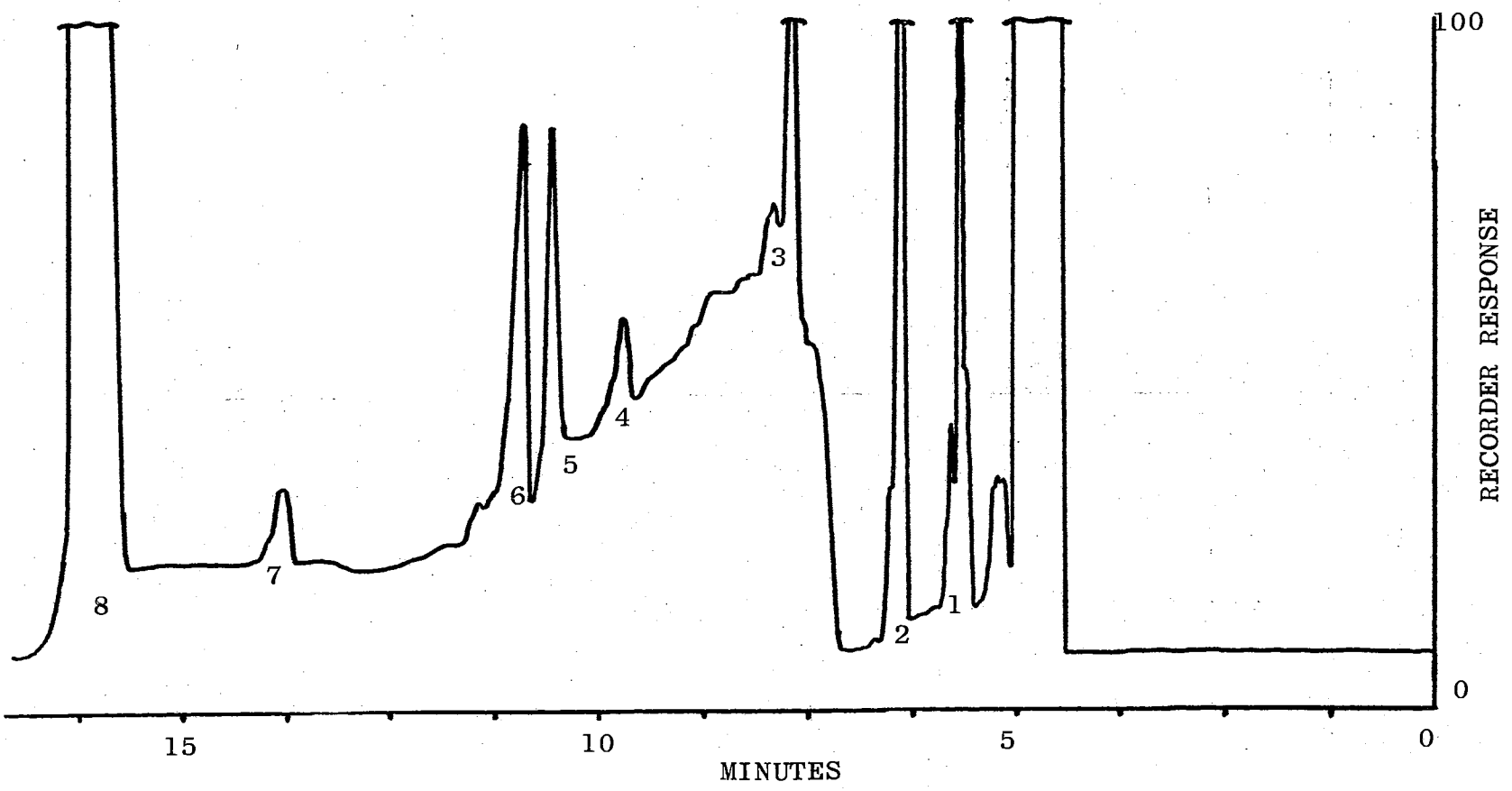

Figure 21. Gas Chromatogram of Volatile Chemicals from Novomessor cockerelli

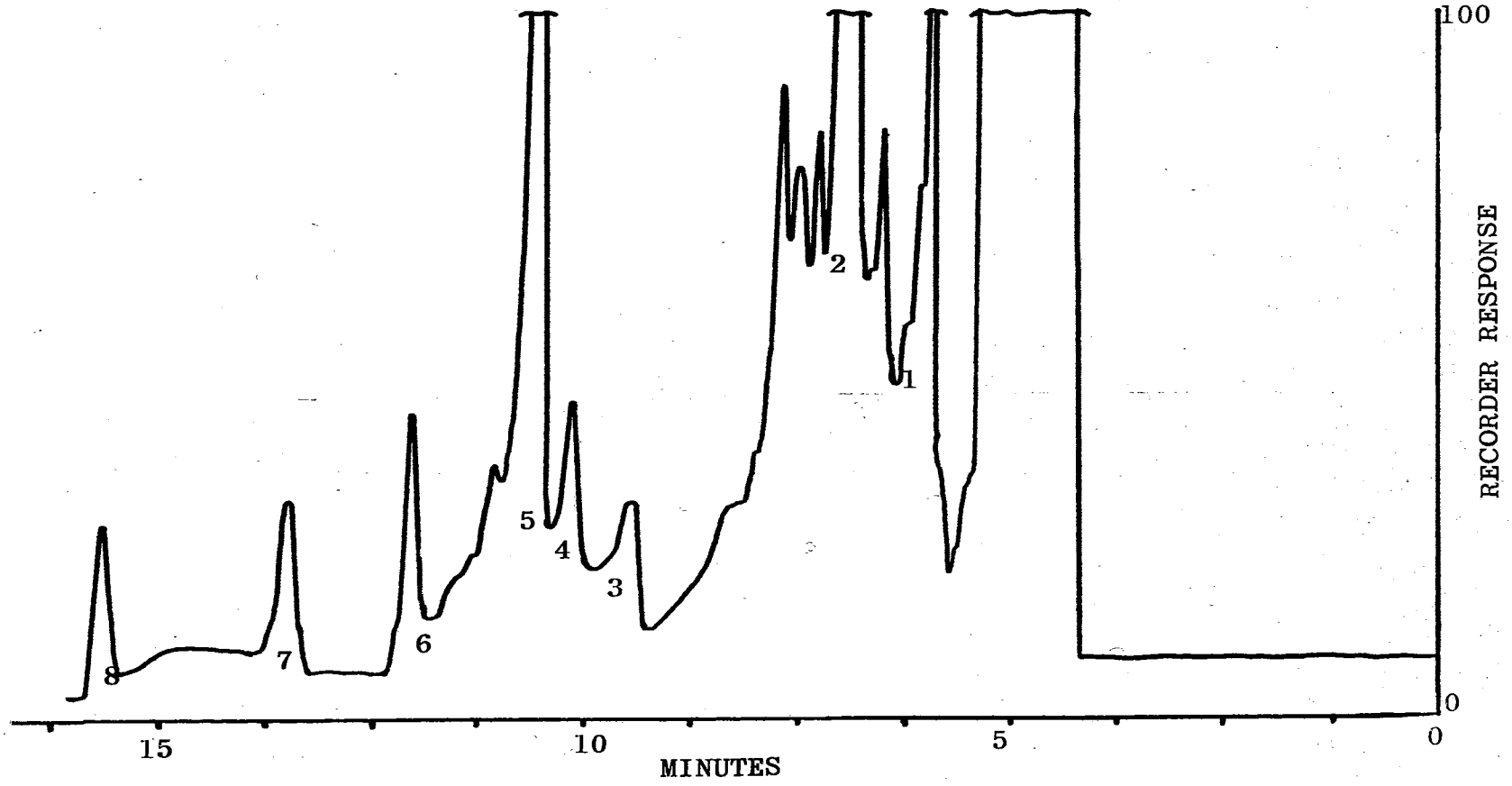

Figure 22. Gas Chromatogram of Volatile Chemicals from Pogonomyrmex barbatus

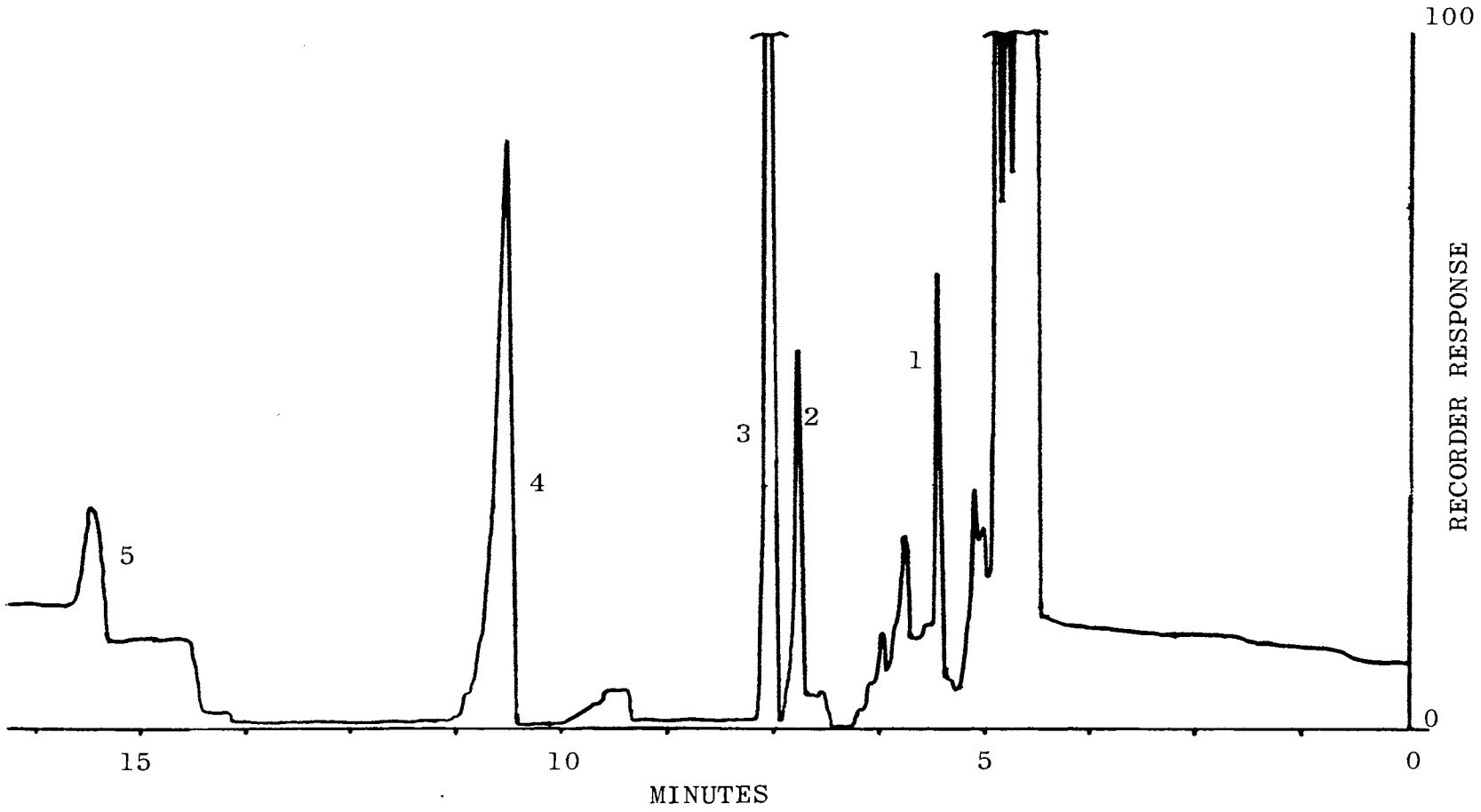

Figure 23. Gas Chromatogram of Volatile Chemicals from Acromyrmex Versicolor

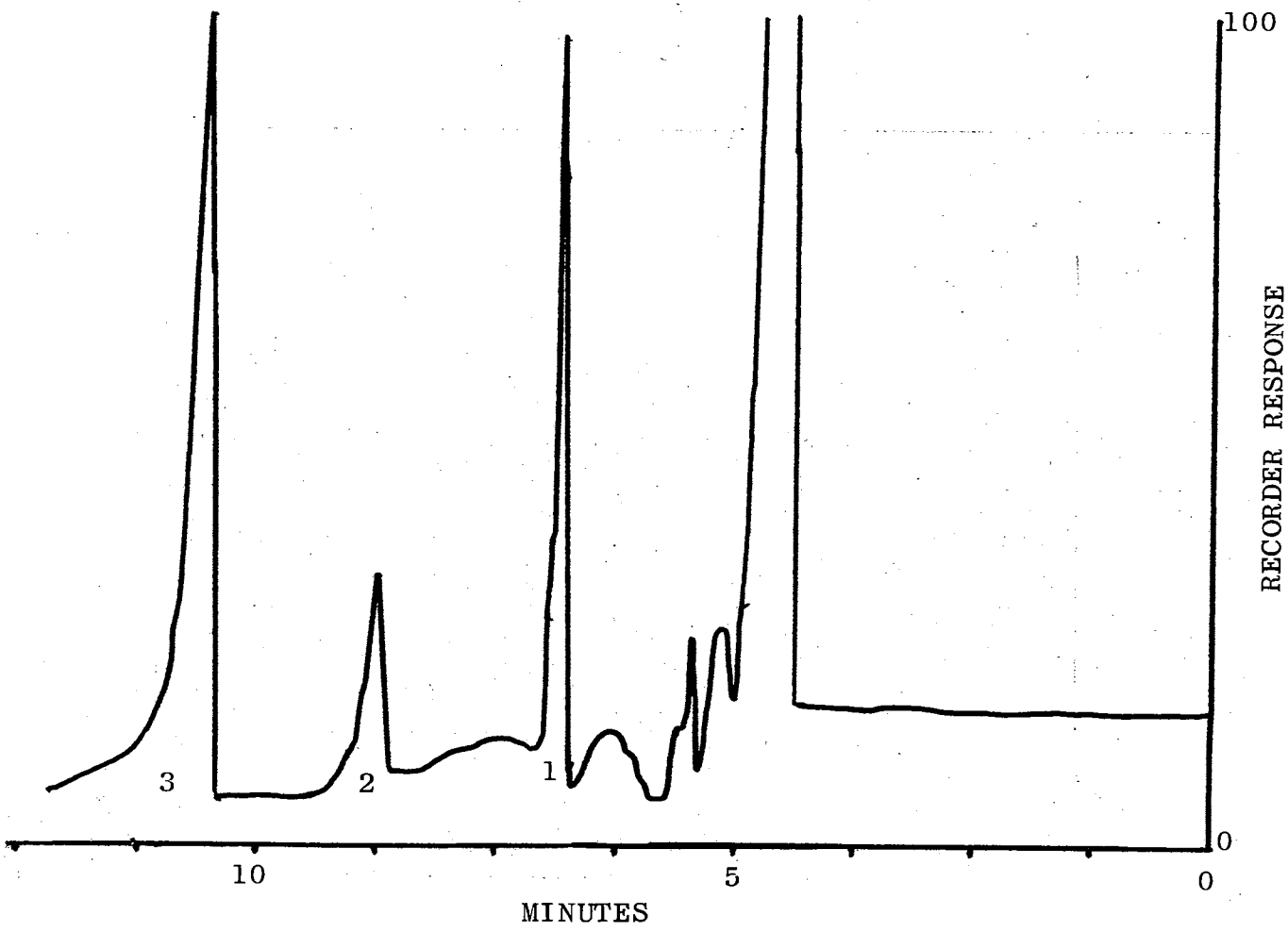

Figure 24. Gas Chromatogram of Volatile Chemicals from Trachymyrmex septentrionalis

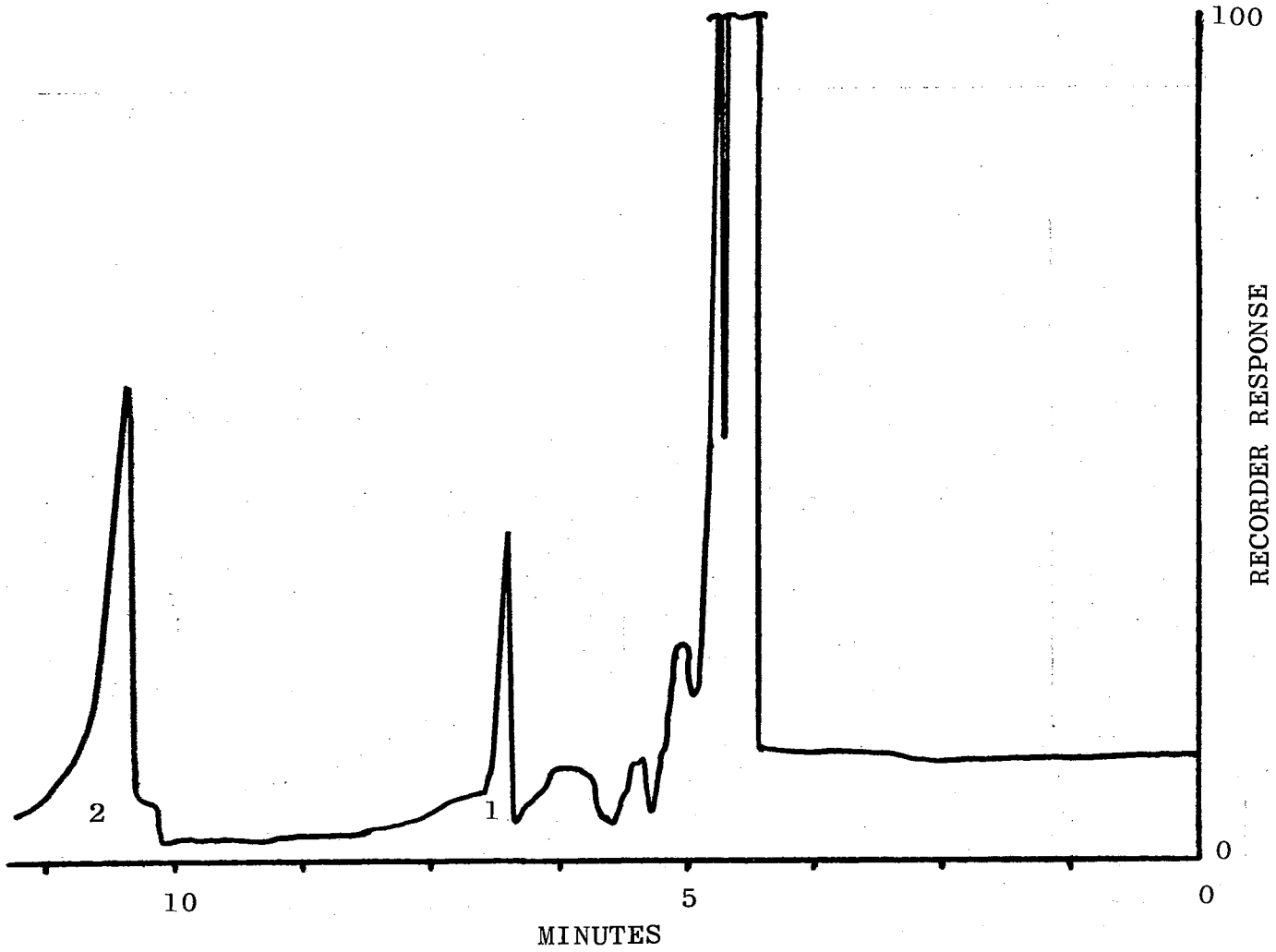

Figure 25. Gas Chromatogram of Volatile Chemicals from Pheidole dentata minor workers

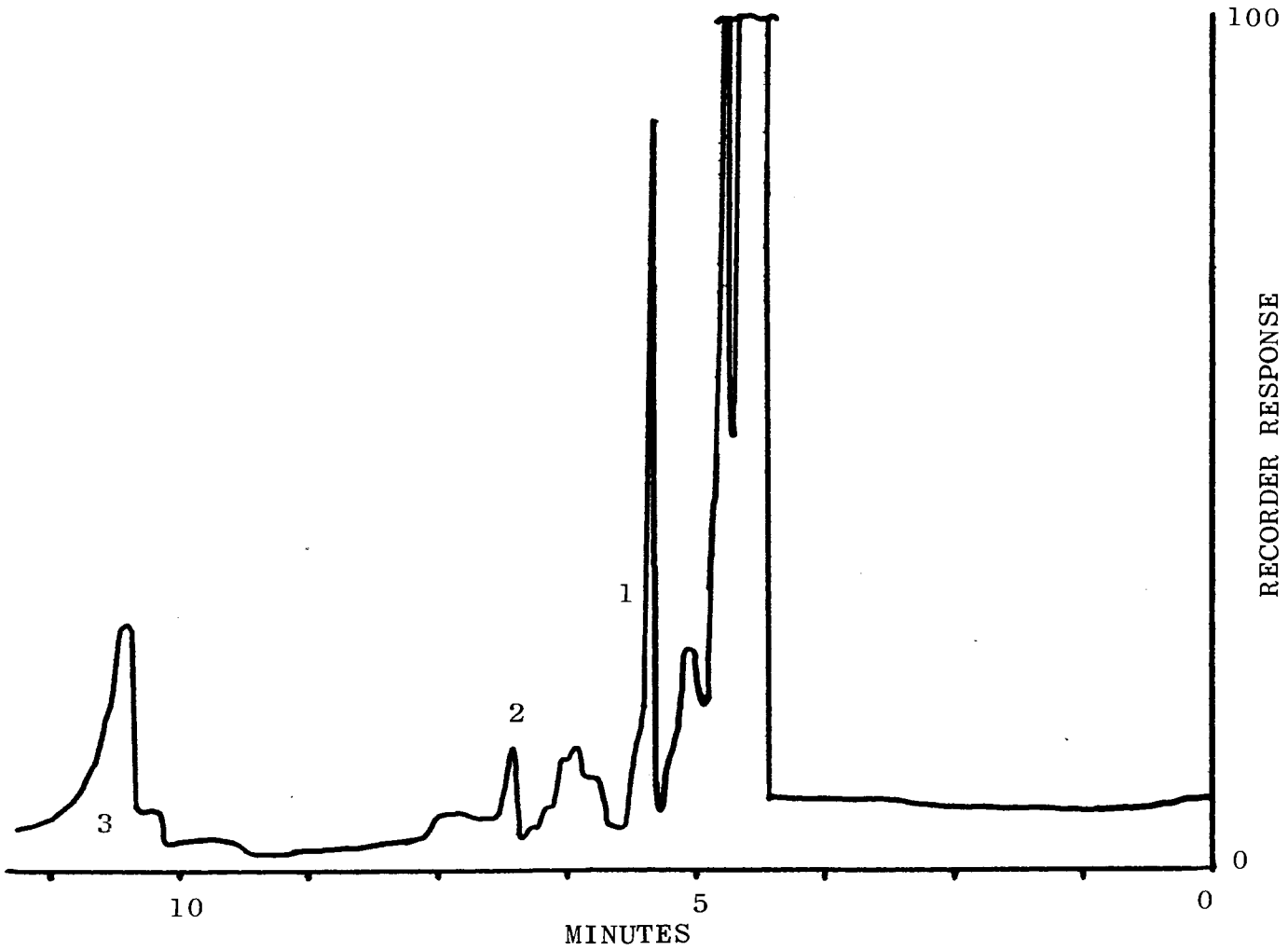

Figure 26. Gas Chromatogram of Volatile Chemicals from Pheidole dentata major workers

TABLE V

INTERNAL STANDARD-PEAK RATIOS FOR

SEVEN ANT SPECIES

Peak	1	2	3	4	5	6	7	8
N. cockerelli	.533	.606	.726	.920	1.00	1.036	1.308	1.515
P. barbatus	.560	.655	.902	.964	1.00	1.143	1.280	1.488
A. versicolor	.518	.647	1.000					
T. septentrionalis	.646	.826	1.000					
D. pyramicus	.484	.668	.747	.923	1.00			
F. foetida	.651	.876	.921	1.000				
P. dentata minor worker	.646	1.000						
P. dentata major worker	.528	.647	1.000					

TABLE VI

REPRODUCABILITY OF INTERNAL STANDARD-PEAK RATIOS FOR *F. FOETIDA*

Observation	Peak 1	Peak 2	Peak 3
1	.648	.877	.931
2	.648	.879	.920
3	.647	.859	.905
4	.651	.876	.923
5	.655	.881	.923
6	.655	.881	.923
Mean	.651	.876	.921
Variance	.004	.009	.027

formed by plus or minus two standard deviation of the mean, will be the result of a different chemical.

The ratio variation is small. The interval formed by plus or minus two standard deviations of the mean for the internal standard ratio of peak one in Table VI is 0.643 to 0.659. The smaller the interval, the greater the probability that two peaks on different chromatograms are the result of two different chemicals.

Table VII contains data on the iridodial isomer variation in Dolichoderine ants. Each has one isomer of at least 80% of the total iridodial concentration. The major isomer is different for each of the three species studied.

Discussion

This study was intended as a preliminary investigation to determine if the technique of gas chromatography might have some merit in ant taxonomy. This first approach was to see if clearly distinct species had chromatograms which were characteristic for that species and whether they were repeatable. The chromatograms obtained do seem to be both species characteristic and repeatable. However, before this technique may be used effectively, the seasonal, geographic, caste and nest variability of the volatile chemicals will need to be studied.

It is necessary to employ an internal standard for three reasons: (1) Gas chromatograph operating

TABLE VII

GAS CHROMATOGRAPHY ANALYSIS OF THE

SILYL ESTERS OF THE IRIDODIOLS

The column and conditions were as follows: 0.02 in. x 450 ft capillary column lined with Monsanto OS-138 (a six-ring polyphenyl ether, bis m - (m - phenoxyphenoxy) phenyl ether) at 120° C and 30 psi helium. A minor peak of less than 12% is represented by the letter "A", a major peak greater than 80% is represented by the letter "B".

	Retention times of Silyl ether (min)	I. pruinosa (%)	D. pyramica (%)	T. sessile (%)
α-Iridodial	50	A	Trace	B
β-Iridodial	60	A	B	A
γ-Iridodial	61	A	A	A
δ-Iridodial	55	B	Trace	A

conditions may fluctuate from analysis to analysis. (2) The column characteristics vary from column to column even when coated with the same substrate. (3) Gas chromatographs of different design often have different chromatographic characteristics. For an internal standard to be most effective, it must be one with a retention time similar to the retention times of the chemicals to which it will be compared. The operating conditions should vary as little as possible between analyses.

It is very important that the temperature used to vaporize the ant volatile chemicals be kept constant. Failure to control this variable will cause qualitative differences in the chromatograms. The temperature of the loop was kept relatively low (200^{o} C) in this experiment in order to simplify the chromatograms since with higher loop temperatures, larger molecules will be vaporized and detected.

Unfortunately, two chemicals with identical retention times cannot be distinguished by gas chromatography alone. Many chemicals have identical retention times on a given column. However, if a mass spectra can be obtained for the peaks in question, one may be able to determine if the peaks are the result of one or two chemicals.

Even though there is still much more work to be done on this technique, there appears to be evidence that it

can give valuable information both on ant classification and phylogeny..

CHAPTER V

FREE AMINO ACIDS AS A TAXONOMIC TOOL

Although much work has been done on the taxonomy of ants, considerable confusion still exists at the species level regarding designations of many species and subspecies (Creighton, 1950). Most of the confusion rests upon the scarcity of adequate characters with which to distinguish different species.

Classification in the troublesome groups is often based upon highly variable characters, often size, color, or the number of hairs on a particular body region. This has led to situations where integrades between different named species commonly occur and where, in some cases, differently colored "subspecies" may nest side by side with no apparent isolating mechanisms. In these situations where morphological characters have proved inadequate, new taxonomic characters are certainly needed.

Insects characteristically have large quantities of free amino acids in their bodies. The free amino acid patterns have been used effectively as taxonomic tools by Micks (1955), Ball (1953), Saxena et al. (1965) and Simpson et al. (1959) on mosquitoes, Hemiptera, and various aquatic invertebrates, respectively. However,

free amino acids were found to have no taxonomic significance by Schaefer and Wallace (1967) in sawfly larvae.

This study was undertaken to determine if free amino acids might provide helpful taxonomic characteristics in the study of ants.

Experimental Methods

The procedure for thin layer chromatography was that of Heathcote and Jones (1965). The glass plates were coated with MN cellulose and the samples chromatographed two-dimensionally with the following solvent systems: (1) isopropyl alcohol, formic acid, water: 40-2-10; (2) tertiery butyl alcohol, methyl-ethyl-ketone, concentrated ammonium hydroxide, water: 50-30-10-10.

Two-dimensional paper chromatograms were run on Number 1 Whatman paper using the following solvent systems: (1) 2-butanol, 3% ammonia: 150-60; (2) 2-butanol, formic acid, water: 150-30-20. One dimensional paper strips were run with the first solvent of the Heathcote and Jones method (1965).

The ninhydrin used for spraying or dipping the chromatograms was prepared by dissolving 1 gram of ninhydrin in 940 ml of acetone and 50 ml of water. They were then dried at 60^{o} C for 30 minutes. The solution used for for extracting the spots was 900 ml of 95% ethanol and

260 ml of water with enough cupric sulfate added so that its final concentration was .05 mg/ml.

Paper strip chromatograms were read with a paper strip densitometer operated manually. The optical density values obtained were then plotted.

The samples were prepared for thin layer and paper chromatography by the following method. The ants were weighed and then homogenized in a glass hand homogenizer with 9 volumes of water. An equal amount of 20% TCA was added to each and centrifuged for 15 minutes at 5,000 rpm. The supernatant was extracted twice with three volumes of ether and then twice with three volumes of chloroform. The aqueous phase was dried in an oven at 60^{o} C and redissolved in a known amount of distilled water.

The ants were prepared for amino acid analyzer analysis as follows. About 0.1 grams of ants were weighed and then homogenized in a glass hand homogenizer in 9 volumes of 3% sulfosalicylic acid. This was centrifuged for 15 minutes at 5,000 rpm and the supernatant drawn off. The residue was washed twice with 9 volumes of 1% sulfosalicylic acid and centrifuged each time as above. The supernatants were combined. An aliquot of the supernatant was dried at 50^{o} C in an oven, then redissolved in 5 ml of 2.2 pH sodium phosphate buffer. The pH was adjusted to pH 2.2 when necessary with con-

centrated phosphoric acid or sodium hydroxide. An appropriate quantity of this solution was applied to the two columns of a Beckman amino acid analyzer and analyzed.

The ants analyzed by the automatic amino acid analyzer had been kept frozen at -18° C for several weeks. The ants used with paper and thin layer chromatography were freshly collected. The species used in this study were Pogonomyrmex barbatus, P. occidentalis, Tapinoma sessile (Say), Campanotus pennsylvanicus (DeGeer), Dorymyrmex pyrimicus (Roger), Novomessor Cockerelli.

Each replicate for the amino acid analyzer was prepared from a different batch of ants and all replicates were prepared simultaneously.

Results

There were 26 ninhydrin positive spots visible after thin layer chromatography of extracts of Pogonomyrmex barbatus. Many of these were subsequently identified using standard amino acids on thin layer and paper chromatography. The identified amino acids from P. barbatus are: leucine, isoleucine, valine, methionine, phenylalanine, tyrosine, proline, alanine, glycine, serine, histidine, lysine, glutamic acid, glutamine, aspartic acid, asparagine, arginine. The following were suspected from automatic amino analyzer data: taurine, -alanine, -aminobutyric acid, ornithine, and ethanolamine.

Although no qualitative species differences were found in the amino acid patterns, there appeared to be noticeable differences in intensities of the ninhydrin positive spots from species to species. Attempts were unsuccessful, however, at eluting the spots from thin layer or paper chromatograms for satisfactory colorimetric analysis.

A one dimensional paper chromatogram of P. barbatus scanned with a densitometer is shown (Figure 27). The chromatogram shows seven more or less well defined peaks. Similar chromatograms were run with replicates for P. occidentalis and Tapinoma sessile and the relative area of each of the peaks calculated. Differences in relative peak area between different species were noted with good agreement within replicates prepared simultaneously. However, when a sample was divided and prepared at different times the procedural variation was great enough to cancel out any species differences.

Figures 28-32 are the chromatograms for the acidic and neutral automatic amino acid column. Quantitative differences in the amino acid patterns between the species are apparent. Arrows on the chromatograms indicate those unknown ninhydrin positive substances with enough interspecific variation to be of taxonomic use. Those amino acids which have been identified are labeled. Table VIII expresses the results in micro moles per gram of ants for each of the identified amino acids. Table IX expresses

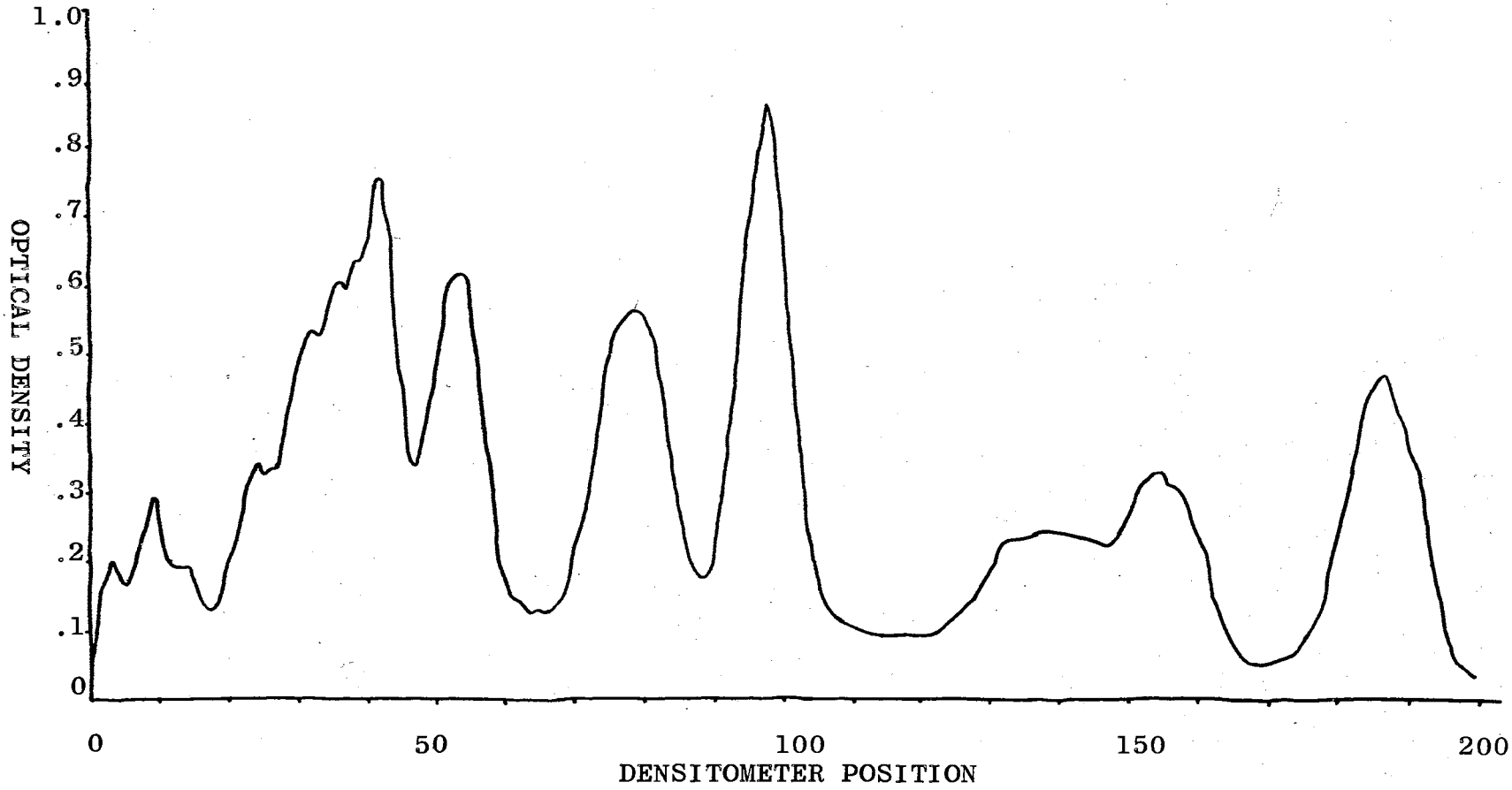

Figure 27. Densitometric Scan of Paper Chromatogram of Ninhydrin Positive Substances from Pogonomyrmex barbatus

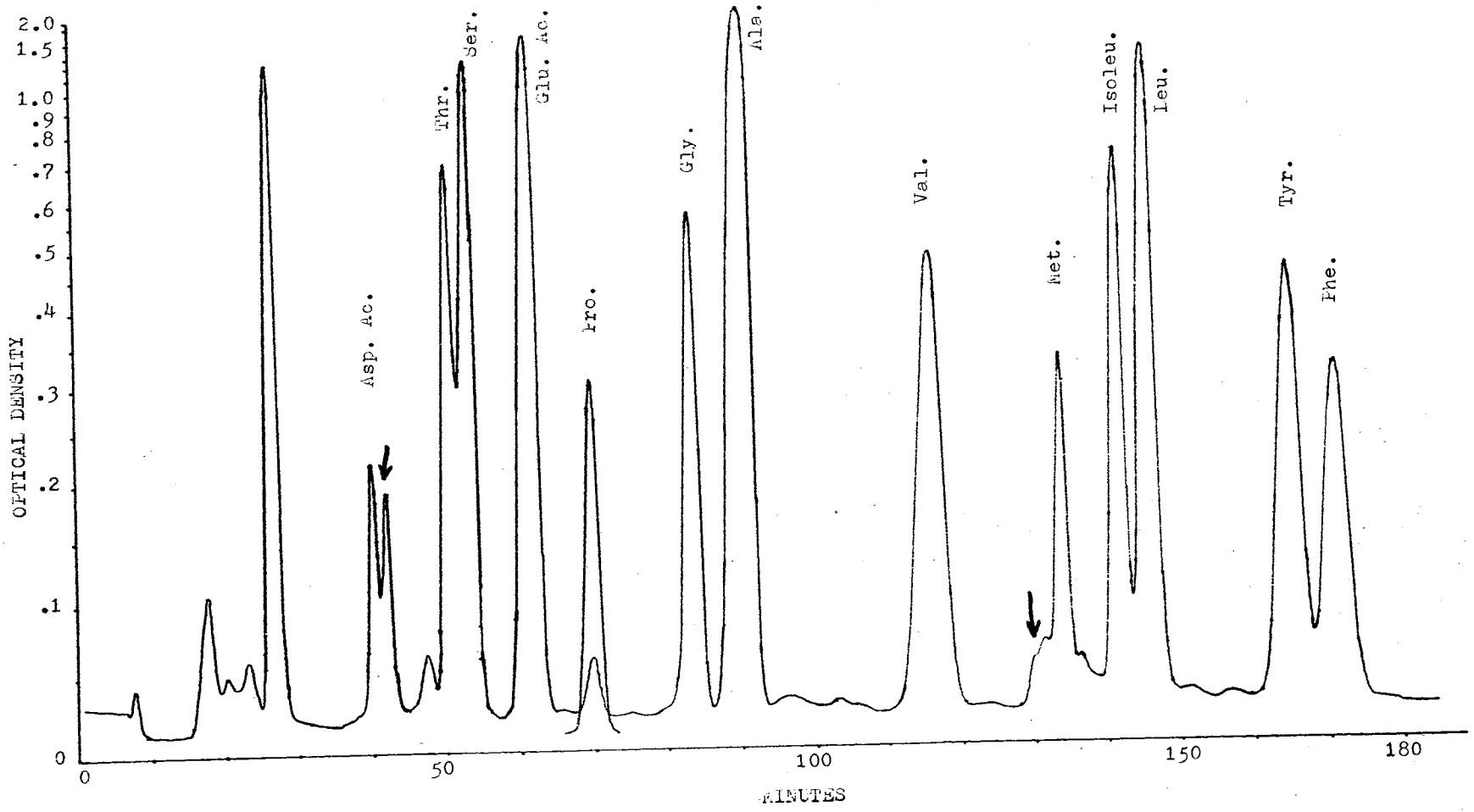

Figure 28. Automatic Amino Acid Analyzer Recorder Tracing of Ninhydrin Positive Substances in Tapinoma Sessile

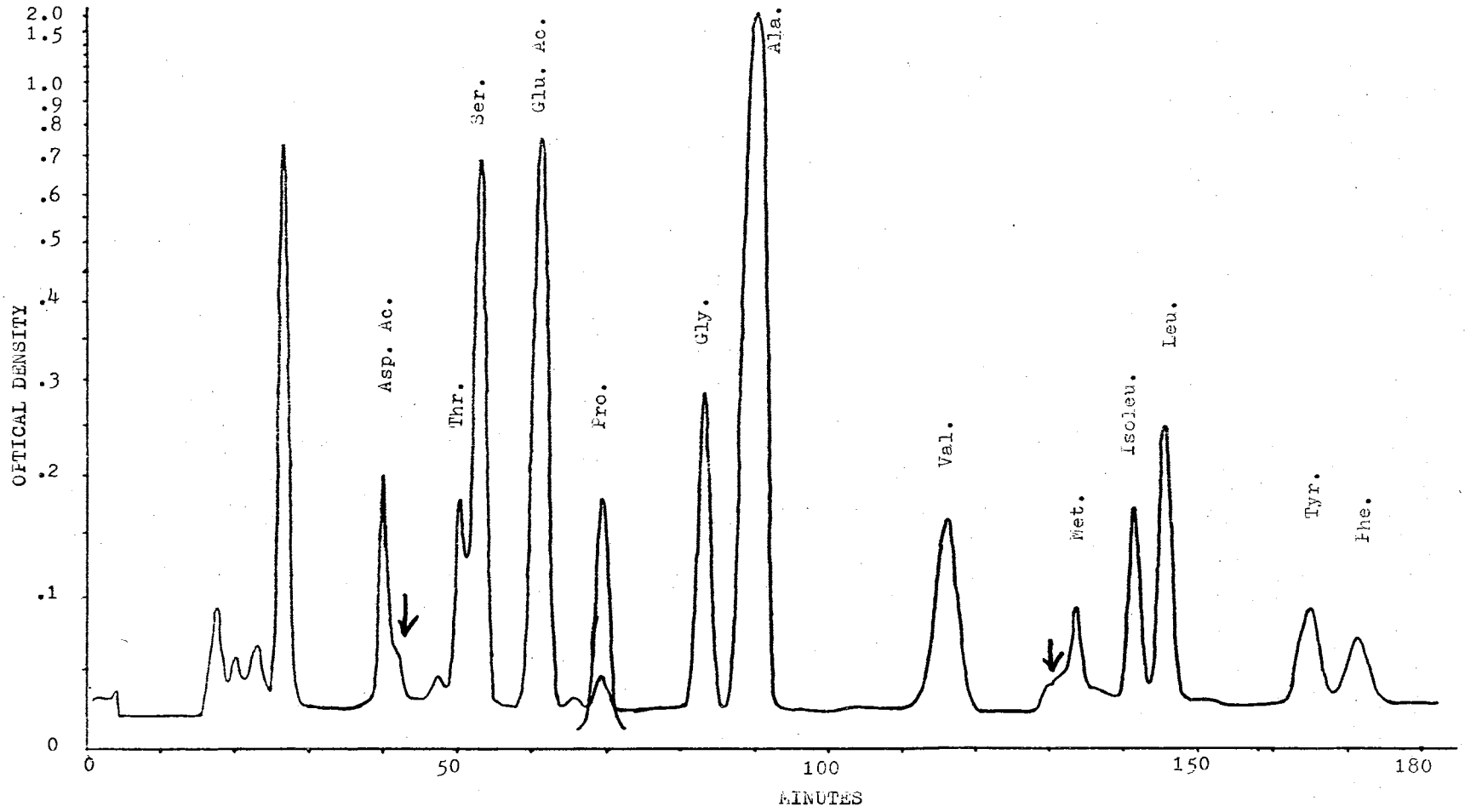

Figure 29. Automatic Amino Acid Analyzer Recorder Tracing of Ninhydrin Positive Substances in Dorymyrmex Pyrimicus

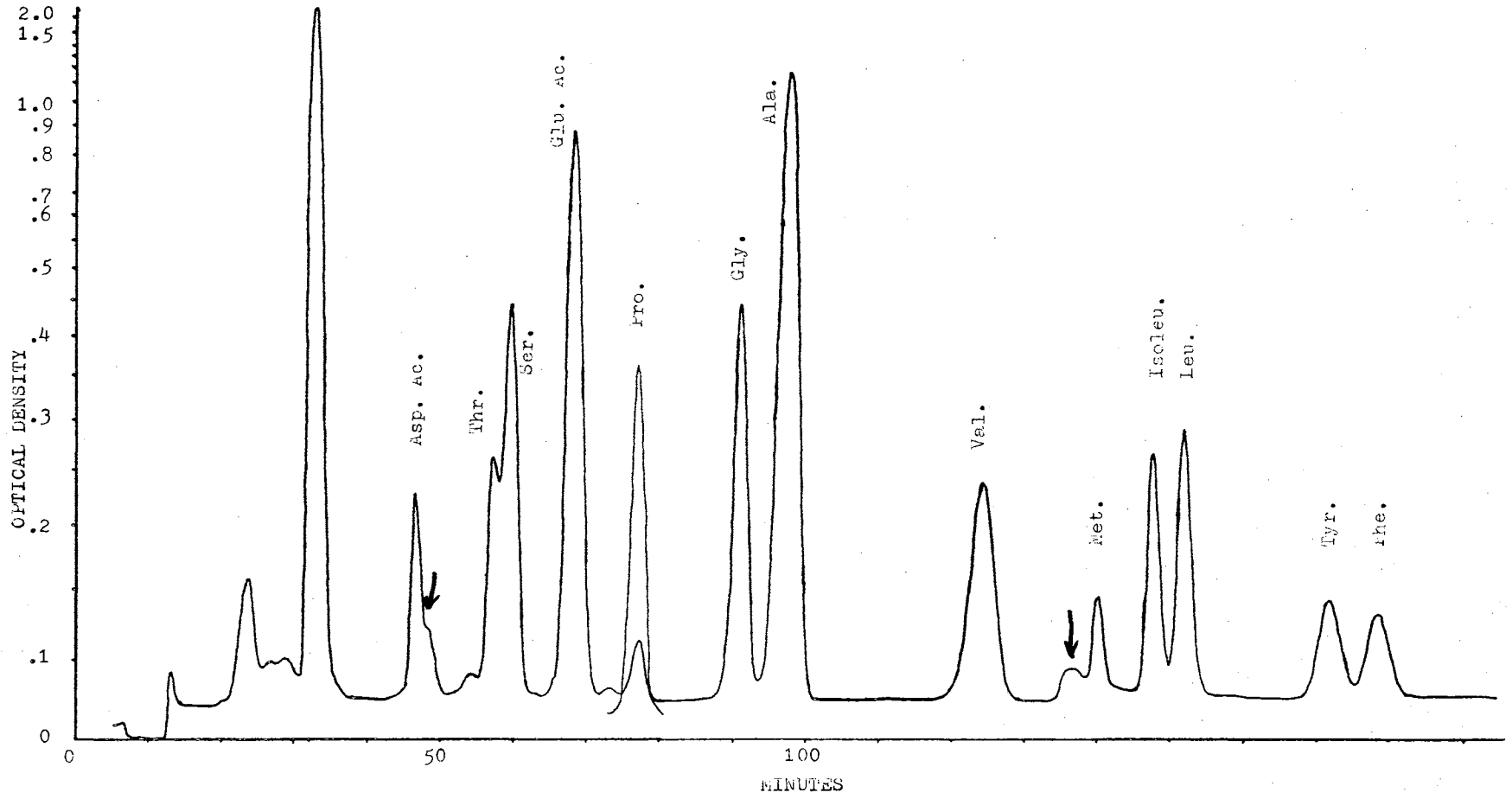

Figure 30. Automatic Amino Acid Analyzer Recorder Tracing of Ninhydrin Positive Substances in Pogonomyrmex Barbatus

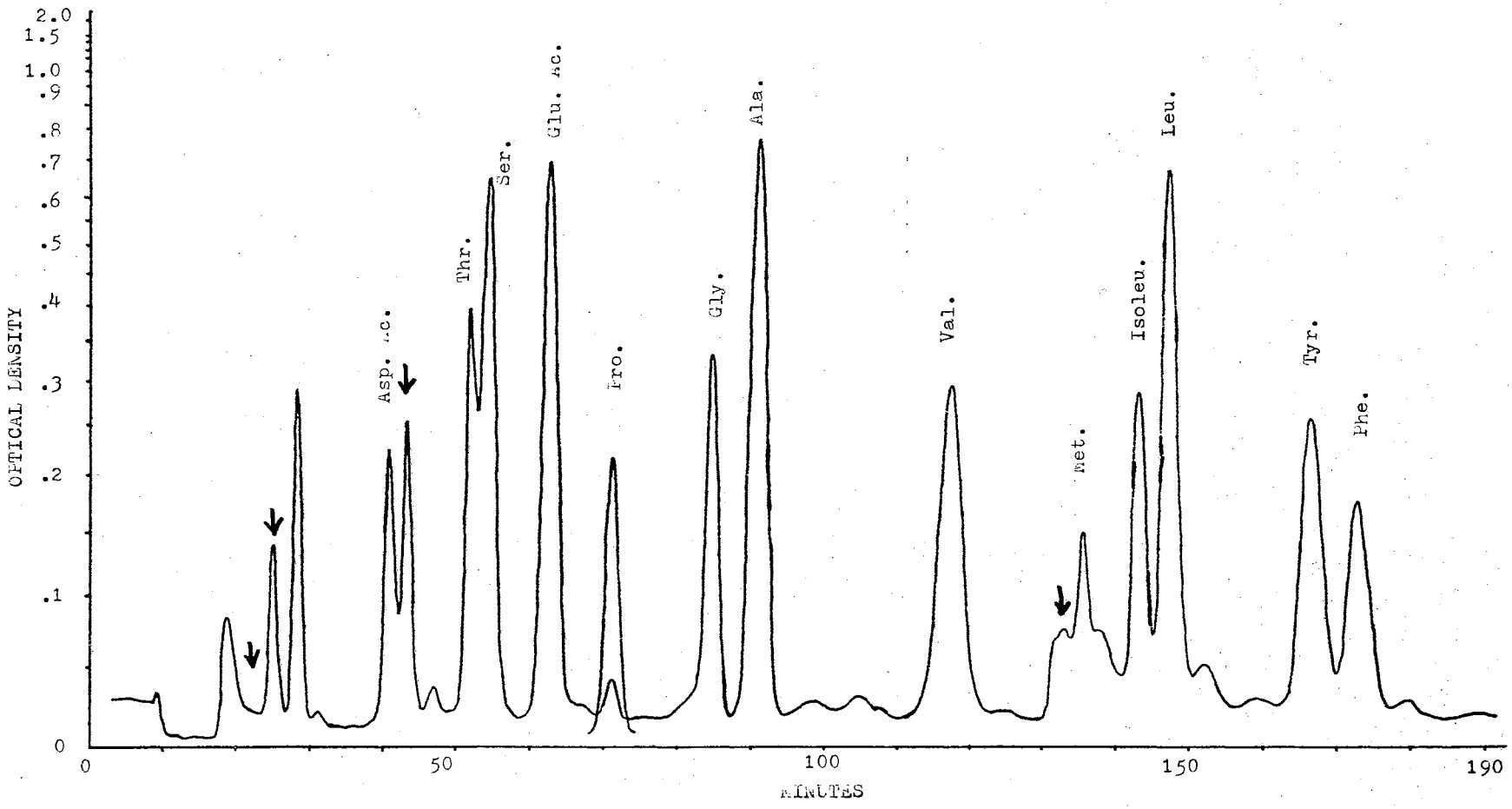

Figure 31. Automatic Amino Acid Analyzer Recorder Tracing of Ninhydrin Positive Substances in Camponotus Pennsylvanicus

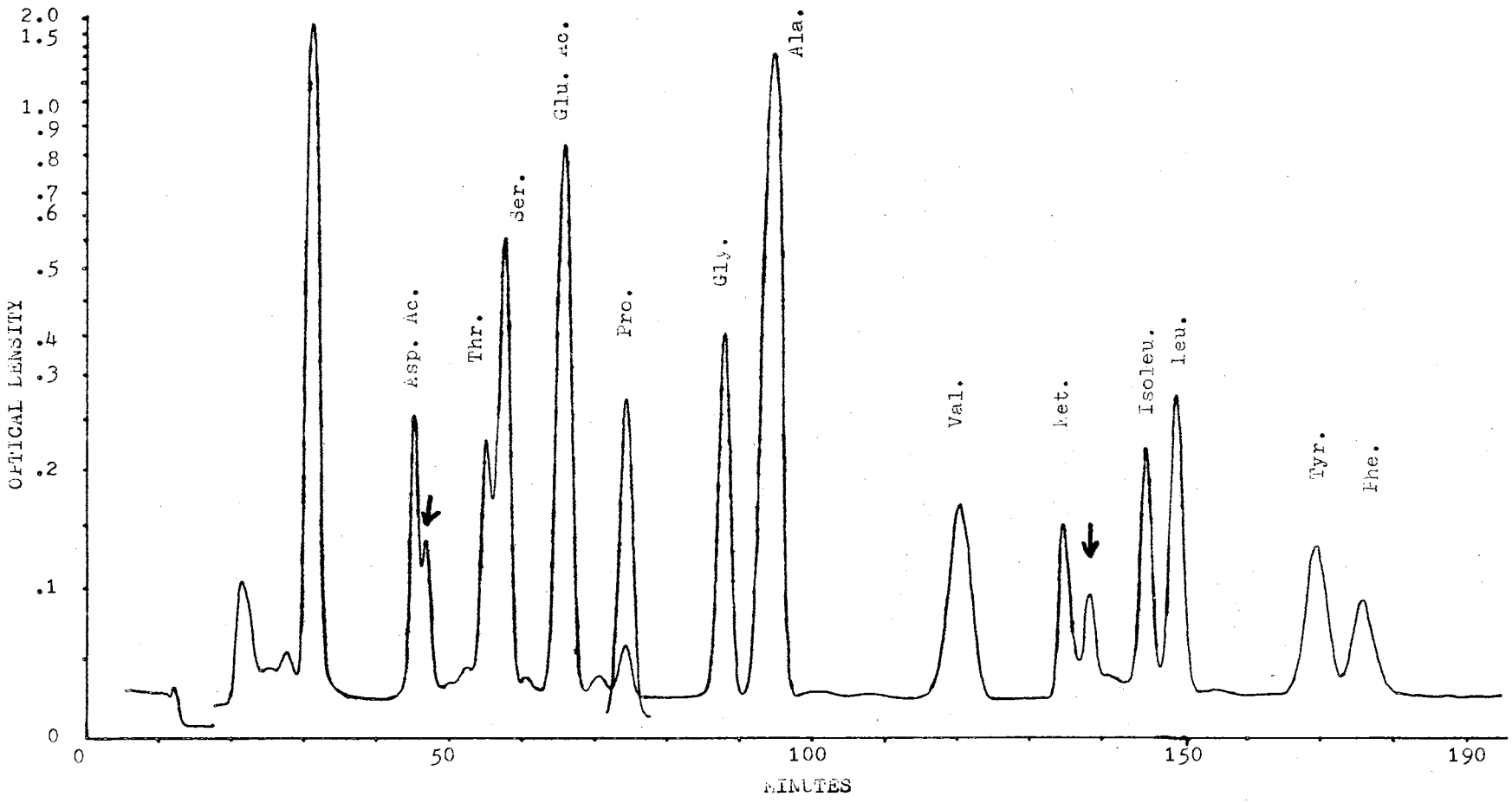

Figure 32. Automatic Amino Acid Analyzer Recorder Tracing of Ninhydrin Positive Substances in Novomessor Cockerelli

TABLE VIII

MICROMOLES OF AMINO ACID

PER GRAM OF ANTS

Amino Acid	P. barbatus[1]	N. cockerelli[2]	C. pennsylvanicus[2]	D. pyrimicus[3]	T. sessile[3]
Aspartic Acid	2.1	6.0	16.4	8.0	9.7
Threonine	11.6	10.7	22.9	11.3	39.4
Serine	21.5	22.6	66.1	33.4	52.1
Proline	79.8	70.4	96.6	52.7	85.2
Glutamic Acid	41.5	40.4	65.1	47.0	84.7
Glycine	20.0	18.1	31.0	17.3	31.5
Alanine	73.9	74.8	77.4	132.8	133.4
Valine	16.6	14.4	50.0	17.0	49.4
Methionine	3.4	3.7	14.0	4.3	13.0
Isoleucine	9.3	9.4	23.2	8.5	30.0
Leucine	11.6	13.4	60.8	13.8	67.9
Tyrosine	6.1	9.4	34.6	8.2	41.5
Phenylalanine	5.5	6.6	26.4	5.7	30.7
Ammonia	138.8	143.9	255.0	107.8	138.3
Lysine	10.5	11.6	41.1	13.5	38.5
Histidine	13.3	7.8	16.6	9.1	20.8
Arginine	41.6	29.8	42.7	33.8	50.2

[1]one replicate

[2]average of two replicates

[3]average of three replicates

TABLE IX

MICROMOLES OF AMINO ACID DIVIDED BY

THE MICROMOLES OF GLUTAMIC ACID

FROM EACH SPECIES

Amino Acid	P. barbatus	N. cockerelli	D. pyrimicus	C. pennsylvanicus	T. sessile
Aspartic Acid	.50	.15	.18	.25	.12
Threonine	.28	.26	.24	.34	.47
Serine	.52.	.56	.71	1.02	.61
Proline	1.90	1.74	1.12	1.48	1.00
Glycine	.48	.45	.37	.48	.37
Alanine	1.79	1.86	2.88	1.19	1.57
Valine	.40	.36	.36	.77	.58
Methionine	.08	.09	.09	.22	.15
Isoleucine	.22	.23	.18	.36	.36
Leucine	.29	.33	.29	.92	.80
Tyrosine	.15	.23	.18	.53	.49
Phenylalanine	.13	.16	.12	.41	.36
Lysine	.25	.18	.29	.63	.46
Histidine	.32	.19	.19	.26	.25
Arginine	1.00	.74	.72	.66	.59

the data as the amount of each amino acid divided by the amount of glutamic acid.

There appear to be free amino acid characteristics for each of these species which would allow the separation of any of five ant species studied from the other four. There are several points at which the data for Tapinoma sessile differs from the other species. For instance, the high leucine content separates T. sessile from all but C. pennsylvanicus. The former's much higher alanine content would easily separate it from C. pennsylvanicus. D. pyrimicus may be separated from the four other species by its very high alanine to glutamic acid ratio. As was pointed out above, the high value for leucine separates C. pennsylvanicus from all but T. sessile and it can be separated from T. sessile by its much lower content of alanine.

P. barbatus and N. cockerelli are the most difficult to separate. It is possible that they might be separated by their differences in quantities of aspartic acid and arginine. There are several points at which they may be distinguished from the other species.

Discussion

Sacena et al. (1965) arranges biochemical characters into two categories: (1) Similarities and differences in the properties of chemical constituents that are function-

ally identical in different animals, and (2) similarities and differences in the distribution of various chemical constituents among different animals." The first category undoubtedly offers the most information about genetic homology, especially the nucleotide sequence of DNA and the amino acid sequence of enzymes and other proteins (Wilson and Kaplan, 1963). However, this information is not likely to be available for any number of chemicals for many years. The chemotaxonomic data currently available is that in the second category.

This preliminary study has demonstrated that the free amino acids in ants when properly quantitated may offer important taxonomic data. These data can be most effectively used at the species level in conjunction with morphological and other chemical characters.

Certainly no phylogenetic relationships may be inferred from this free amino acid data. Brown (1967) has pointed out the errors which can be made by attempting to establish phylogenetic relationships at the generic and higher levels upon the basis of the quantities of normal metabolites present in animals. However, the amino acid data does establish 16 new points of comparison between the five species studied.

More work is particularly needed to establish the variability of the free amino acids from different localities. The similarities in amino acid patterns

between closely related species as well as the effects of morphological clines within a species upon the amino acid patterns should also be investigated.

Although Ball (1953) reported qualitative differences in the amino acid pattern of three species of Culex mosquitoes, no qualitative differences were observed in the ants studied. It is worthwhile to note that many times chemicals have been reported absent from an organism only to have a refinement in technique demonstrate its presence. Although there is always the possibility that some amino acid unique to a group of ants might be found, it seems likely that most comparisons will be on a quantitative basis.

CHAPTER VI

SUMMARY

The alarm pheromone of Pogonomyrmex barbatus has been identified as 4-methyl-3-heptanone. The alarm substance threshold concentration was determined to be 9×10^{13} molecules/ml of air. Pogonomyrmex ants were able to detect 4-methyl-3-heptanone in smaller quantities than any of 14 other ketones tested.

The hydrocarbons of Novomessor cockerelli were investigated. N. cockerelli was found to contain a series of saturated, straight chain hydrocarbons from C-13 to C-19. Branched isomers of decane and octadecane were also found. The glandular origin of these hydrocarbons is in doubt.

The volatile chemicals from several species of ants were chromatographed to determine their possible value as taxonomic characters. Qualitative differences were apparent between all of the species studied. The necessity of using an internal standard was shown.

Ninhydrin positive substances were investigated for possible chemotaxonomic usefulness. Qualitative differences

between species were difficult to determine, but quantitative differences were apparent and repeatable.

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