COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

INSTITUTE OF ANIMAL AND FOOD SCIENCES

DIVISION OF FOOD RESEARCH

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FINAL REPORT ON "IDENTIFICATION OF COMPOUNDS RESPONSIBLE FOR 'IODOFORM-LIKE' OFF-FLAVOURS IN PRAWNS AND OTHER EDIBLE CRUSTACEANS"

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1 SUMMARY

A wide variety of off-flavour problems associated with crustaceans have in the past been termed "iodoform-like". Work carried out in this Laboratory showed that the existing off-flavour problems were complex and that the term "iodoform" could only be applied to a proportion of off-flavoured material being brought onto the consumer market. The "iodoform" problem appeared to be principally associated with the endeavour prawn, *Metapenaeus endeavouri* of Western Australia and only occasionally with the large king prawn, *Penaeus plebejus*, caught off the eastern coast of Australia.

A problem of greater commercial significance occurs with the deep-sea royal red prawn, *Hymenopenaeus sibogae* and the shallow water sand-lobster, *Ibacus peronii*, both of which have been described as having an "iodoform" off-flavour. However when first confronted with the problem in this Laboratory, the flavour was described as resembling that of garlic with a pronounced metallic after-taste. It has been shown that this garlic-metallic off-flavour is multicomponent, and that in the case of the royal red prawn it does not arise as a consequence of poor handling techniques. In contrast, two minor off-flavours described as rotten onion and moth-ball were directly associated with microbial spoilage resulting from poor handling procedures. Consequently, certain recommendations have been made that will alleviate some of the off-flavours and give rise to a product of greater visual appeal.

2 INTRODUCTION

Flavour problems encountered both overseas and locally

Australian prawns, in common with prawns and shrimps caught throughout the world, are subject to an "iodoform" off-flavour from time to time[1]. When such prawns are strongly tainted, the odour can be detected in the fresh, uncooked flesh. Prawns intended for export are not rejected if they contain the "iodoform" flavour in a minor degree, since, according to the United Nations draft specifications this flavour must be "very intense" before it constitutes a defect.

Another off-flavour is encountered in mediterranean waters, a garlic flavour associated with the red prawn, but this flavour is highly esteemed by the local inhabitants, who prefer to cook these prawns before heading, to conserve the garlic flavour[2].

In recent years deep-sea trawling off the N.S.W. coast has led to significant catches of the royal red prawn, *Hymenopenaeus sibogae*, along with smaller quantities of the carid prawn, *Plesionika sp.*, and to a lesser extent, the red prawn, *Aristeomorpha foliacea*. These three species have at all times a strong garlic-metallic off-flavour. Although the royal red prawn has considerable commercial potential, we have noticed that the quality of royal red prawns sold through the Sydney fish markets is extremely poor, and this is reflected in the generally low price that these prawns yield compared with shallow water species.

However, studies with consumer panels showed that the royal red prawn was just as acceptable as the more highly prized eastern king prawn, when both were presented as crumbed cutlets, although the latter was preferred when both were presented as cold, boiled prawns[3].

The carid prawn has not, as yet, achieved economic importance, although its use as a major component in prawn and sea food pastes has been examined and the feasibility of a glass packed product in direct competition with overseas potted shrimp has also been tested.

The red prawn is of no commercial importance because of its limited occurrence, its large head to tail ratio and its very strong garlic-metallic off-flavour.

The same off-flavour encountered in these deep-sea prawns is found in the shallow water sand-lobster, *Ibacus peronii*, which at present is being established as a product for export. However the garlic component is at least ten times more intense in the sand-lobster than in prawns[4].

3 ASSESSMENT OF THE PROBLEM

In 1978 Mr John Last of this Division visited Markwell Fisheries Pty. Ltd. at Tweed Heads to obtain information concerning the occurrence of the "iodoform" flavour. The only flavour problem encountered was associated with royal red prawns, and although the factory was familiar with the "iodoform" flavour occasionally found in eastern king prawns, they had not encountered this flavour in any eastern king prawns recently. In addition to royal red prawns, the local sand-lobster, *Thenus orientalis*, sometimes presented a strong off-flavour.

In the same year Mr Last also visited Western Australian fishing industries and learnt that the endeavour prawn, *Metapenaeus endeavouri*, was affected throughout the entire fishing season by the "iodoform" off-flavour. This problem appeared to be worst in Exmouth Gulf. Other species of prawns caught in Western Australian waters are the tiger, *Penaeus esculentus*, and the western king, *Penaeus latisulcatus*, which are sold for approximately twice as much as endeavours and are caught in far greater numbers. When storage space is limited, skippers may throw endeavours overboard. However, Mr Last was told that if cooked fresh there was generally no off-flavour in endeavour prawns, although they were otherwise nearly always off-flavoured.

A visit made by Mrs Freeman to Western Australia resulted in information which conflicted with that obtained by Mr Last. She was informed that although the "iodoform" flavour was prevalent in endeavour prawns, it was present intermittently throughout the season and not continuously. Furthermore heading of these prawns on board boat made no difference to the presence of this off-flavour, which was present as soon as the prawns were landed on deck.

Assessment of the off-flavour problem with the royal red prawn caught off the eastern coast was made by visits to Markwells at Tea Gardens, N.S.W., the Newcastle Fishermens' Co-op and to the Sydney Fishmarkets.

Mr Ron Taylor at Tea Gardens claimed royal red prawns did have a distinctive garlic flavour which occurred in the fresh state; however the major off-flavour problem with these prawns arose from their poor handling during storage by the fishermen on board boat and in transit to the markets and by processors in the factories. Mrs Freeman confirmed this by visits to fish markets and retail outlets in Sydney where the quality of royal red prawns sold is extremely poor.

Mr Keith Thornton of Newcastle Fishermens' Co-op also confirmed that royal red prawns presented storage problems to the fisherman and still received poor public acceptance even with careful treatment.

The garlic off-flavour problem associated with the sand-lobster *Ibacus peronii*, has been attributed to *bis*-methylthiomethane, the same component found in deep-sea prawns. However, this off-flavour problem differs from the problem occurring in the prawns in a number of aspects. Firstly this problem is only present intermittently. Secondly, when present the garlic flavour is so intense that the flesh is completely inedible. Thirdly, only a small percentage of the batch, at times as low as 8-10 individuals out of 200 may be affected, but the intensity of the offensive odour is so great that the whole batch may be condemned by Fishing Inspectors.

4 EXPERIMENTAL PROCEDURE

a) Isolation

All initial observations of odour components were made using headspace collection techniques to avoid the identification of artifactual off-flavours. Volatile components were collected from homogenates produced from 100 g of crustacean flesh and 150 cm^3 of 24% aqueous sodium chloride. They were first adsorbed on to porous polymer traps at $40^{o}C$, and then transferred by the "freezing on" technique to a glass SCOT column. The column was then placed in a temperature-programmed gas liquid chromatogram (g.l.c.), operated with helium as the carrier gas. In the initial runs the effluent from the column was monitored by the nose to detect the compounds responsible for the off-flavours, which were assigned to discrete peaks on the g.l.c.

In subsequent, duplicate runs these components were trapped for further investigation.

Other methods for obtaining volatile components from crustaceans used in these investigations included steam distillation under reduced pressure and the combined continuous distillation-solvent extraction method of Likens and Nickerson[5] using freon 11 as solvent. Alternately more simply, by a Likens-Nickerson extraction of the crustacean homogenates.

b) Method of identification

i) Reaction g.l.c.

Two reaction g.l.c. techniques were employed:

Microhydrogenation - Samples of the individual compounds collected from the g.l.c. effluent were hydrogenated at $160^{o}C$ for 3 min on a bed of PtO_2 (c. 1 mg) contained in a 100-µl Microcap.

Microozonolysis - Samples of the individual compounds (c. 10 µg) were ozonized in a film of butyl butanoate contained in a 100-µl Microcap.

ii) Mass spectrometry (m.s.)

Low resolution mass spectra were recorded on an Atlas CH_4 mass spectrometer coupled to a laboratory-designed gas chromatograph using the column and operating conditions previously described[6]. High-resolution mass spectra were recorded on a Varian MAT-311A mass spectrometer.

iii) 'H n.m.r. spectrometry

Spectra were recorded on a Brucker CXP 100 spectrometer fitted with a 'H microprobe head. Samples (5-50 μg) were trapped from the g.l.c. effluent in a 100-μl Microcap (1.42 mm o.d.), the area about the trapping zone was cooled in solid carbon dioxide. The Microcap was sealed at one end and 99.96% C_6D_6 (8 μl) was introduced. The solution was centrifuged to the closed end of the tube and the other end was immediately sealed. The sealed Microcap was inserted into a PTFE sleeve fitted to an inverted n.m.r. tube (5 mm o.d.). Depending on the size of the sample, between 1,000 and 12,000 scans (1 to 12 h) were required to obtain a definitive spectrum.

c) Quantitive estimations

Concentrations of components were estimated from their peak areas on the chromatogram, by relating them to the peak areas on chromatograms obtained using known amounts of the compound.

d) Synthesis

Once identified, the flavour components were synthesized following fairly standard procedures (see Section 5).

5 RESULTS AND DISCUSSION

a) Identification of compounds and their flavour characteristics

Analysis of volatile components obtained from prawns and sand-lobsters led to the isolation of components with a garlic flavour (compound A), a mushroom flavour (compound B) and a metallic flavour (compound C), Fig. 1 and Table 1. Compound A had a molecular formula of $C_3H_8S_2$ and showed major ions in its low-resolution mass spectrum at m/e 61 ($M - CH_3S$), m/e]08 ($C_3H_8S_2$) and at m/e 35(CH_3S) (Fig. 2). The spectrum was identical to the published spectrum of *bis*-(methylthio)-methane[7]. The 'H n.m.r. spectrum was consistent with this structure (Fig. 3). Synthesis of *bis*-(methylthio)-methane by the reaction of 37% aqueous formaldehyde with methyl sulfide in the presence of gaseous hydrogen chloride[8], gave a material identical in all respects to the natural product. Cooking of freshly minced crustacean flesh with *bis*-(methylthio)-methane gave a material with a distinctive garlic flavour. The concentration of *bis*-(methylthio)-methane in several species of prawns and sand-lobsters possessing the garlic-metallic flavour is reported in Table 1.

Compound B had a molecular formula of $C_8H_{16}O$ and was in all respects identical with an authentic sample supplied (ORIL).

Compound C had a molecular formula of $C_8H_{14}O$ and showed major ions in its low resolution mass spectrum at m/e 108 ($m - H_2O$), m/e 93 ($m - H_2O - CH_3$), m/e 79 ($m - H_2O - C_2H_5$), m/e 70 ($m - C_3H_4O$) and m/e 57 (Fig. 4). The compound when examined by reaction gas chromatography gave octan-3-ol and octan-3-one on hydrogenation, and formaldehyde and propanal as the major products on ozonolysis. This data indicated that compound C was an octa-1,5-dien-3-ol, a 'H n.m.r. confirmed this, however the complexity of the spectrum (Fig. 5) prevented assignment of the stereochemistry about the C-5 double bond. Synthesis of (5 Z)-octa-1,5-dien-3-ol in five steps (Fig. 6) gave a material identical in all respects to the natural product.

The addition of both of these alcohols in concentrations of 50 μg kg^{-1}

to samples of minced prawns or sand-lobster flesh of bland flavour, followed by cooking at 100°C for 3 min, gave a material with an initial mushroom flavour, and an intense metallic after-taste. When the flesh was also treated with *bis*-(methylthio)-methane at a concentration of 5 μg kg^{-1} (to samples containing both of the alcohols), the cooked material had a garlic-metallic flavour not detectably different from the naturally occurring off-flavour.

A poorly handled consignment of royal red prawns, condemned as spoiled, had a distinct rotten onion off-flavour superimposed on the usual garlic-metallic flavour. The prawns examined had damaged shells, the flesh was discoloured and the product felt slimy. It is our belief that the onion off-flavour was related to microbial spoilage of the product - a result of poor handling techniques.

Analysis of headspace volatiles indicated a component with a rotten onion aroma, compound D, which gave a low-resolution mass spectrum with major ions at m/e 79 (M - CH_3S) and 126 (CH_3SSSCH_3). This spectrum was in agreement with the published spectrum of dimethyl trisulfide[9]. Synthesis of dimethyl trisulfide yielded a product identical in all respects with the natural compound[10].

The addition of dimethyl trisulfide in concentrations of 1-10 μg kg^{-1} to bland samples of minced crustacean flesh, followed by cooking gave a material with a distinctive and unpleasant onion flavour. Addition of this material to prawn flesh at a concentration of 100 μg kg^{-1} (the level found in the prawns under study), rendered the cooked material inedible. Examination of volatile compounds from undamaged royal red prawns showed that dimethyl trisulphide was occasionally present but in concentrations (less than 0.1 μg kg^{-1}) too low for detection of an off-flavour.

b) Distribution of off-flavour components and variations among species

It has been established that the major source of all these off-flavour compounds was the head section, which includes the stomach and digestive glands of the animal. The tails of uncooked prawns were found to be free of *bis*-(methylthio)-methane and contained only traces of the alcohols (Fig. 7 & 8). However upon cooking the animals whole, a significant proportion of these compounds was transferred to the flesh (Fig. 9). In parts of eastern Australia it has been common practice to cook the entire crustacean catch while at sea. Where animals contained significant quantities of these compounds in the "head" section this practice would have resulted in edible flesh becoming contaminated with the garlic-metallic off-flavour. Contrary to popular belief, removal of the mid-gut from the tail plays a negligible role in improvement of the overall taste, however, it does prevent ingestion of the unpleasantly gritty material usually present in the alimentary tract of the prawn (Fig. 10-13).

The occurrence of *bis*-(methylthio)-methane and the two alcohols, oct-1-en-3-ol and (5 Z)-octa-1,5-dien-3-ol in a variety of crustaceans is recorded in Table 1.

c) Possible origin of off-flavour compounds

The identified off-flavours may originate from one or more of the following:

i) Natural components from the crustacean's diet; examination of mud from 200-300 fathoms, the depth at which royal red prawns are caught, has suggested that this is the likely source of oct-1-en-3-ol and (5 Z)-octa-1,5-dien-3-ol.

ii) Metabolites from the crustaceans enzymic processes or metabolites from the action of gut microorganisms. Dimethyl trisulfide is a metabolite produced by spoilage of prawns. Pseudomonas putrifaciens and P. perolens[12] have been shown to produce dimethyl trisulfide when sterile fish muscle is incubated with these bacteria even at $0^{o}C$. The isolation of indole, a known indicator of microbial spoilage[13], at a concentration of 50 $\mu g\ kg^{-1}$ from damaged samples of prawns, but not from undamaged ones, supports the suggestion that the onion flavoured component was derived from bacterial contamination. However, the presence of traces of dimethyl trisulfide in some undamaged prawns indicates it to be a normal metabolite of the prawn; It probably arises by action of gut microorganisms which proliferate in the damaged samples and produce sufficient dimethyltrisulfide to cause off-flavour problems.

iii) Pheromonal compounds associated with the animals' life cycle.

iv) By products produced by bacteria present on the shell and contaminants introduced from fish slime encountered during trawling.

6 RECOMMENDATIONS TO THE INDUSTRY

Investigation into the off-flavour problems in crustaceans has brought to our notice the poor quality of royal red prawns sold through the Sydney fish markets and local outlets.

Royal reds are an excellent prawn but they do require more careful handling than is usually given to other species. They are thin shelled and fragile, so that, to limit damage the prawns should be carefully and quickly sorted from the remainder of the catch and gently washed to remove any mud or slime that could harbour bacteria. They should then be immediately transferred into suitable containers such as semi rigid plastic barrels, which are topped-up with refrigerated sea water (R.S.W.) at about -2°C, and immersed in R.S.W. It has been found that royal red prawns can be held in R.S.W. for 3-5 days without unduly affecting their quality.

Handling royal red prawns in this manner has three advantages:

i) physical damage is reduced;

ii) the low temperature retards the development of bacteria and the formation of chemical compounds that give undesirable flavours to the prawns;

iii) the R.S.W. in the containers prevents the prawns from coming into contact with atmospheric oxygen, the cause of "black spot".

In the absence of R.S.W., a slurry of sea water and ice is almost equally as effective but as the resulting temperature (about 0°C) is not as low as R.S.W. the effective total holding period will be slightly less.

Storage in ice is definitely not suitable, even for very short periods since there is insufficient protection from air to retard black-spot development. In addition, movement within the ice boxes tends to damage the prawns to the point where quality of the meat is seriously affected.

Royal red prawns should not at any stage be left without the protection of refrigeration because the longer they remain unrefrigerated the greater their tendency to develop off-flavours.

Finally we recommend that royal red prawns be distributed through the wholesale-retail network as frozen peeled or unpeeled glazed tails in appropriate packages with impermeable outer wrappings.

In the case of the sand-lobster, it would appear that the garlic off-flavour arises only in a few individuals in a given catch after death. It is advisable to either keep them alive by covering them with a damp cloth and keeping them relatively cool, or alternately cooking them alive and then storing the cooked product on ice. Further investigations are being carried out to determine the cause of the garlic off-flavour in these creatures.

7 CONCLUSION

Careful handling of royal red prawns results in a high quality product which nevertheless has a distinctive flavour which has been described as garlic-metallic. The appearance of a pronounced onion and mothball aroma is a direct result of faulty handling and can therefore be eliminated by correct procedures.

Recently it has been observed that the garlic flavour associated with the sand-lobster *Ibacus peronii*, differs from that found in prawns. In the sand-lobster the garlic component *bis*-(methylthio)-methane is present in much larger quantities, and appears to form only after death. This off-flavour can be eliminated totally by following the recommendations outlined in section 6 of this report. Work is currently being undertaken to explain this phenomenon as well as the origin of the off-flavour compounds.

8 ACKNOWLEDGEMENTS

We wish to thank Mr T. Gorman, New South Wales Department of Fisheries, and the Sydney Fish Markets for the gifts of prawns and sand-lobsters and Mr A. Ward from Lakes' Entrance Fishermens Co-op for providing us with samples of sand-lobsters. The award of a Fishing Industry Research Grant (to D.J.F.) is gratefully acknowledged.

TABLE 1

Occurrence and concentration of components responsible for off-flavour in some crustacea

Common Name	Zoological Name	Average Concentration μg kg^{-1}		
		bis-(methylthio)-methane	oct-1-en-3-ol	(5 Z)-octa-1,5-dien-3-ol
PRAWNS				
Royal Red	*Hymenopenaeus sibogae*	3	40	49
Red	*Aristeomorpha foliacea*	4	38	44
Carid	*Plesionika sp.*	4	37	41
SAND-LOBSTER				
Balmain bug	*Ibacus peronii*	14	14	11

9 REFERENCES

1. Montgomery, W.A., Sidhu, G.S. and Vale, G.L., *C.S.I.R.O. Food Preservation Quarterly*, 1970, 30(2), 21.

2. Gorman, T.B.S. and Graham, K.J., *National Prawn Seminar, Maroochydore, Queensland*, 1973, 162.

3. Ruello, J.H. and McBride, R.L., *The Fisherman*, 1973, 4(9), 4.

4. Whitfield, F.B., Freeman, D.J., Last, J.H. and Bannister, P.A., *Chem. and Ind.*, 1981, 158.

5. Likens, S.T. and Nickerson, G.B., *Proc. Am. Soc. Brew. Chem.*, 1964, 5.

6. Whitfield, F.B. and Sugowdz, G., *Aust. J. Chem.*, 1979, 32, 891.

7. Sloot, D. and Harkes, P.D., *J. Agr. Food Chem.*, 1975, 23, 356.

8. Böhme, H and Marx, R., *Chem. Ber.* 1941, 74, 1642.

9. Schreyer, N., Dirink, P., Van Wassenhove, F. and Schamp, N., *J. Agric. Food Chem.*, 1976, 24, 336.

10. Nakabayashi, T., Tsurugi, J. and Yabuta, J., *J. Org. Chem.*, 1964, 29, 1236.

11. Miller, A. III, Scanlan, R.A., Lee, J.S. and Libby, L.M., *Appl. Microbiol.*, 1973, 26, 18.

12. Miller, A. III, Scanlan, R.A., Lee, J.S., Libby, L.M. and Morgan, M.E., *Appl. Microbiol.*, 1973, 25, 257.

13. Farber, L., In "Fish as Food" (Ed. G. Borgstrom) Vol. 4, Pt 2., pp. 80 (Academic Press, New York, 1965).

VOLATILE COMPONENTS FROM UNCOOKED SAND LOBSTERS

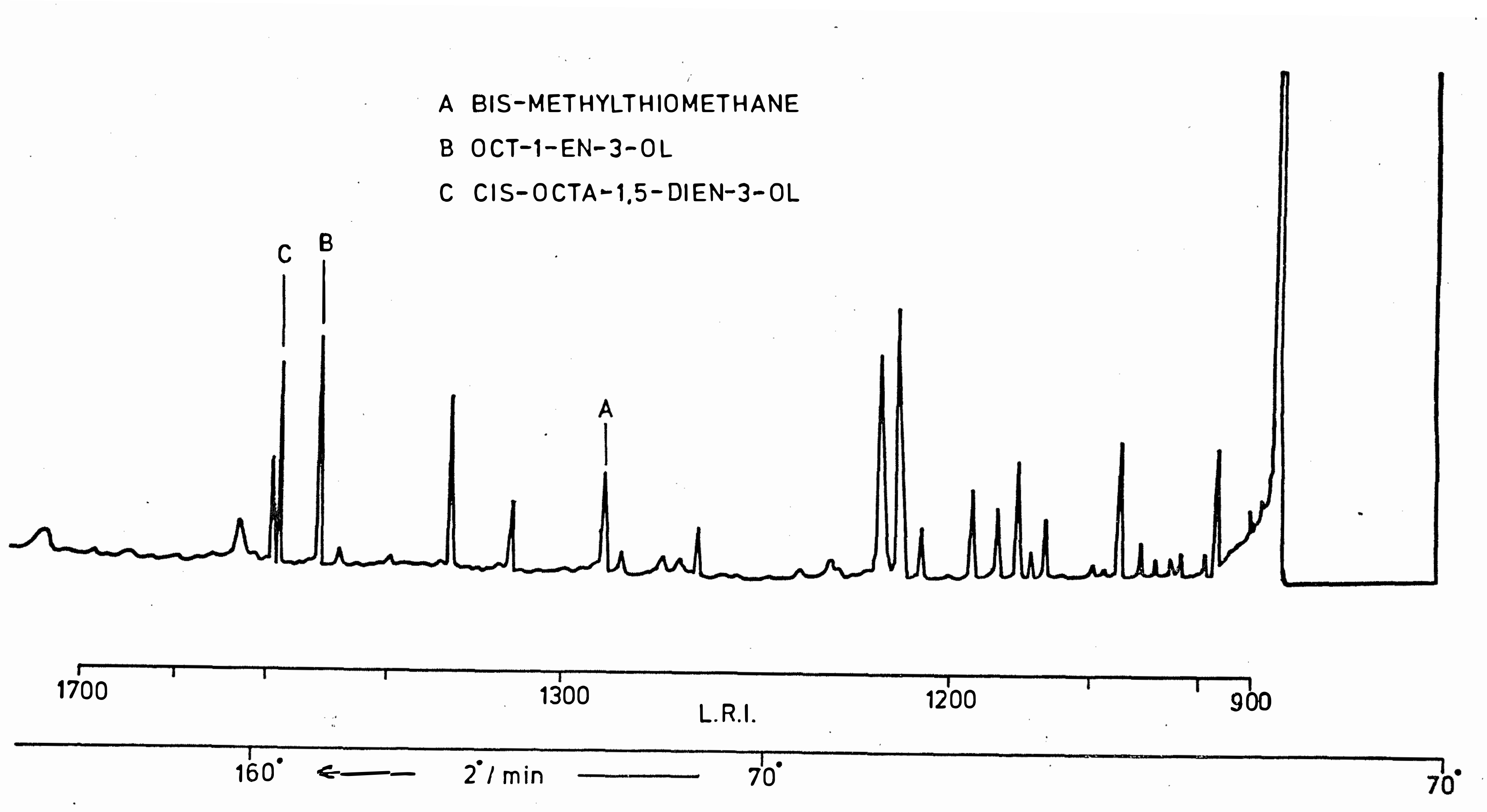


Fig 1

LOW RESOLUTION MASS SPECTRUM OF BIS-(METHYLTHIO)-METHANE

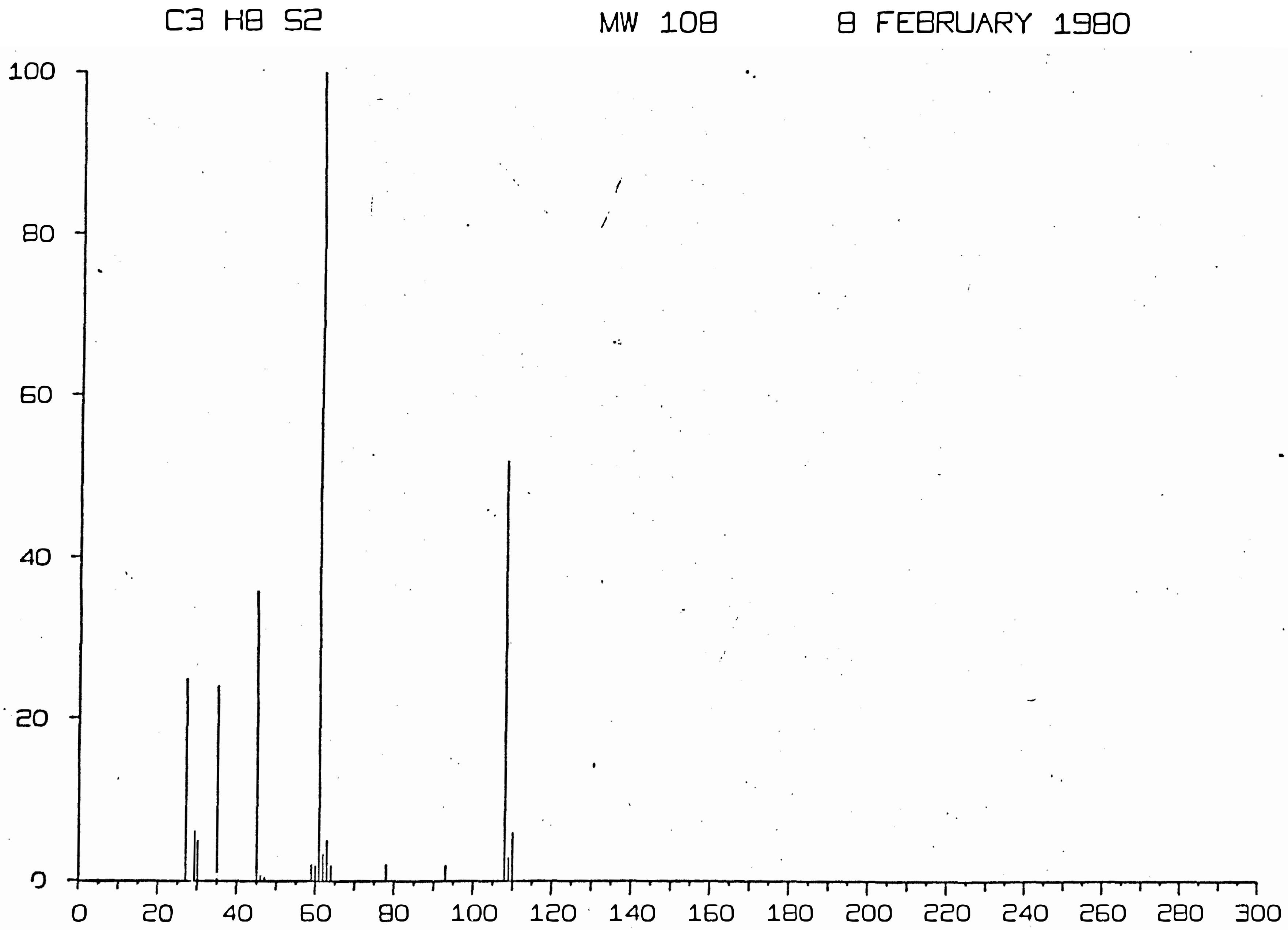


Fig 2

^{1}H n.m.r. SPECTRUM OF BIS-(METHYLTHIO)-METHANE

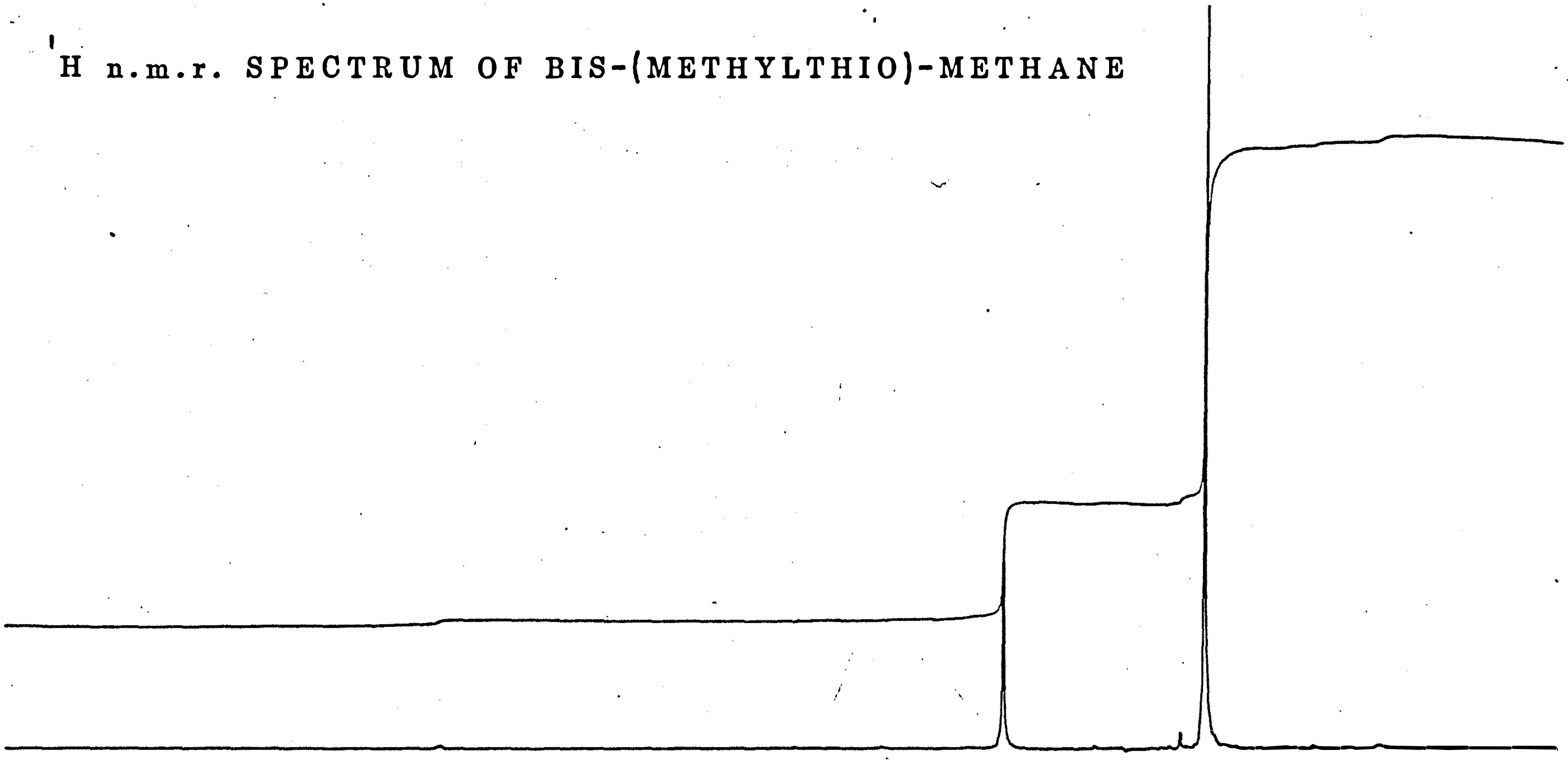


Fig 3

LOW RESOLUTION MASS SPECTRUM OF (5Z)-OCTA-1,5-DIEN-3-OL

C8 H14 O MW 126 21 FEBRUARY 1980

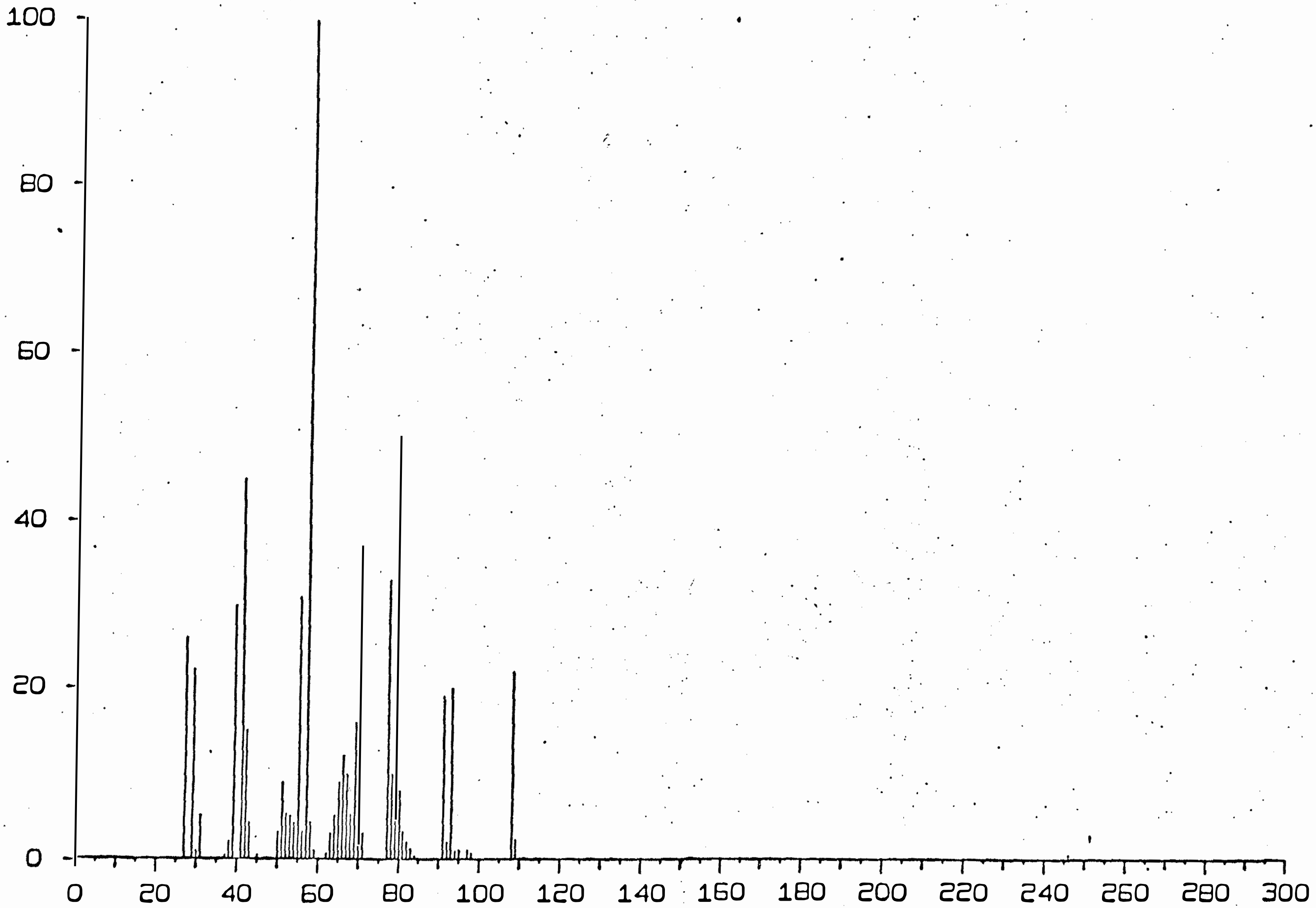


Fig 4

^{1}H n.m.r. SPECTRUM OF (5Z)-OCTA-1,5-DIEN-3-OL

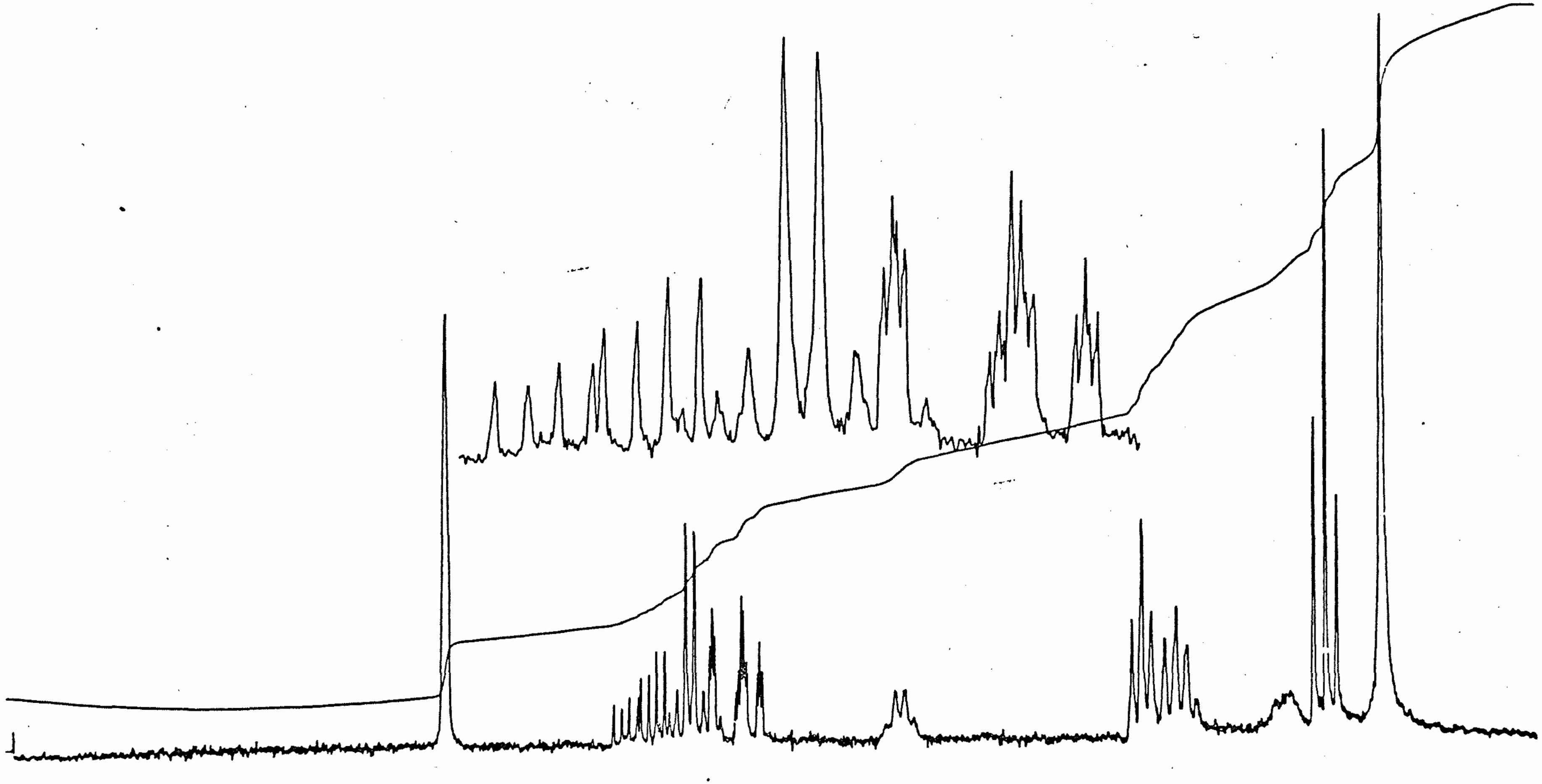


Fig 5

SYNTHESIS OF (5Z)-OCTA-1,5-DIEN-3-OL

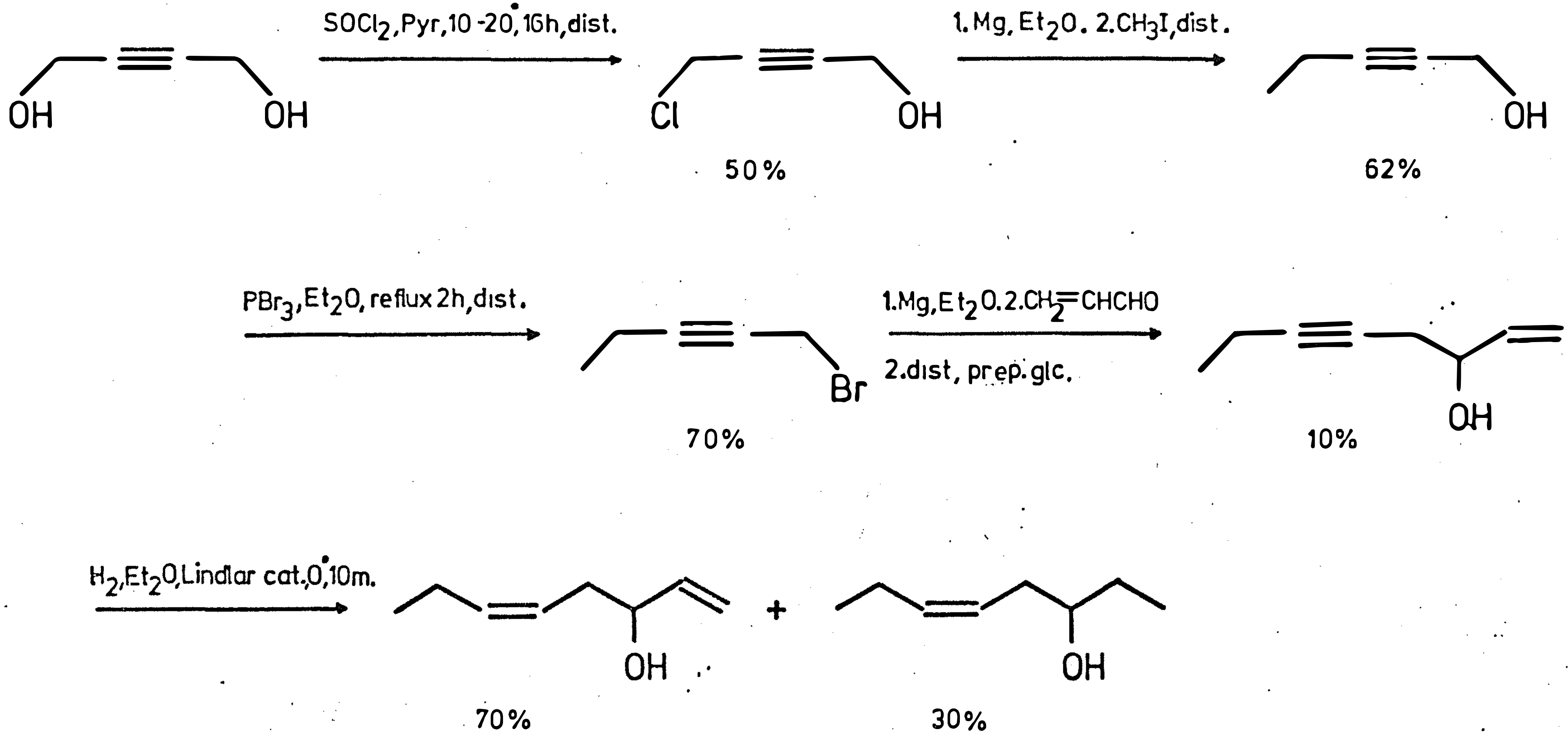


Fig 6

VOLATILE COMPONENTS FROM UNCOOKED PRAWNS

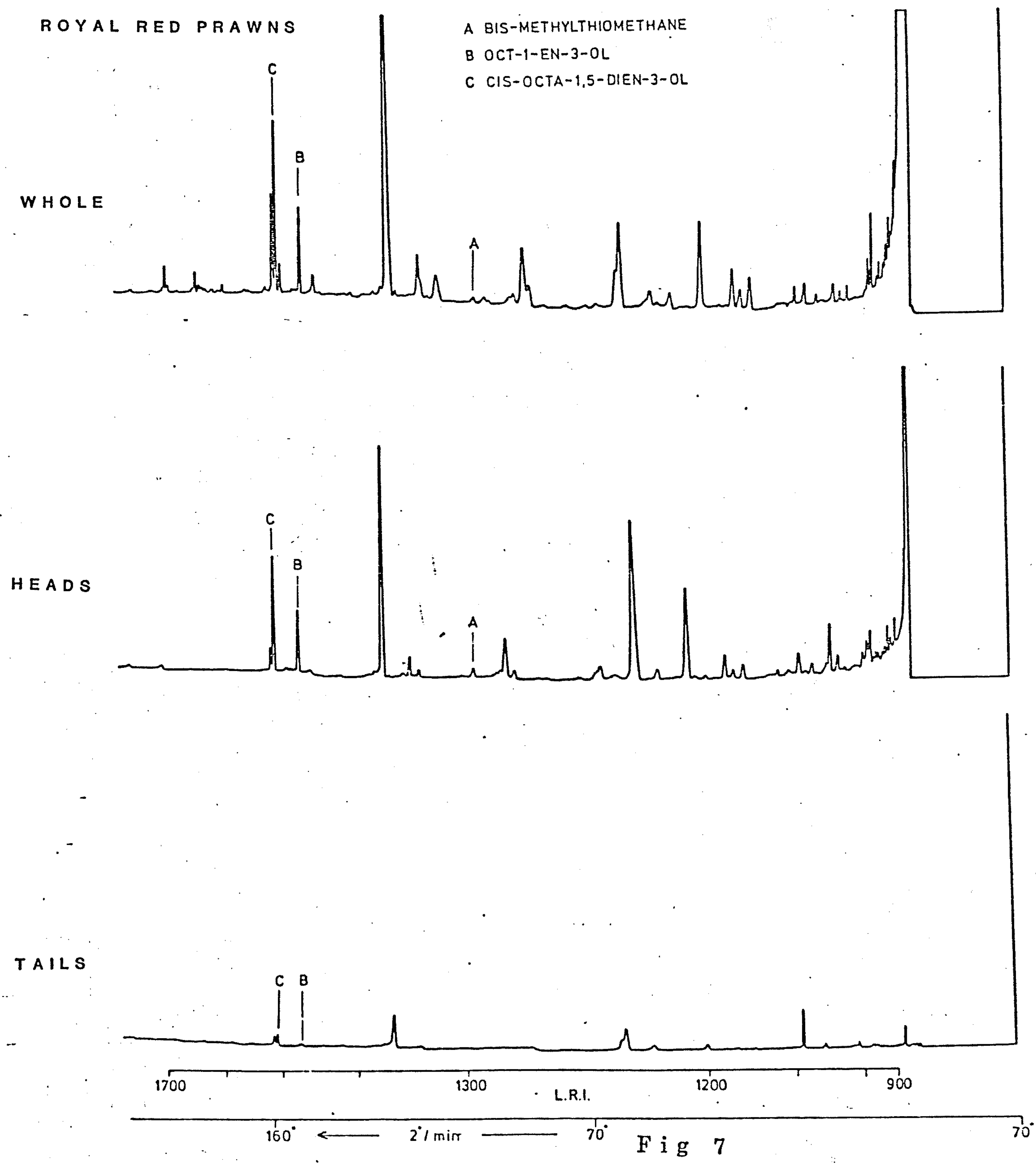


Fig 7

VOLATILE COMPONENTS FROM UNCOOKED SAND LOBSTERS

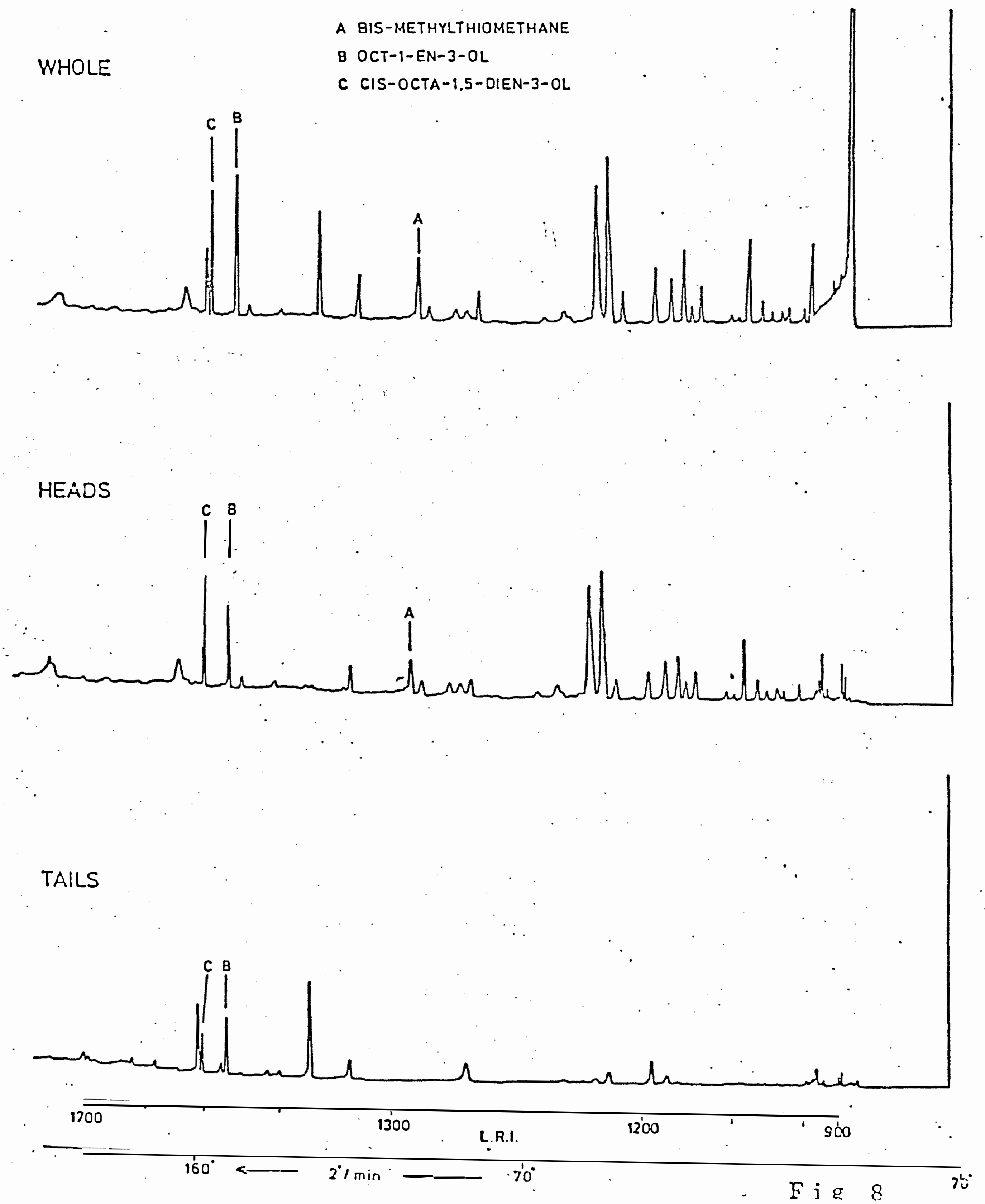


Fig 8

EFFECT OF COOKING ON DISTRIBUTION OF PRAWN VOLATILES

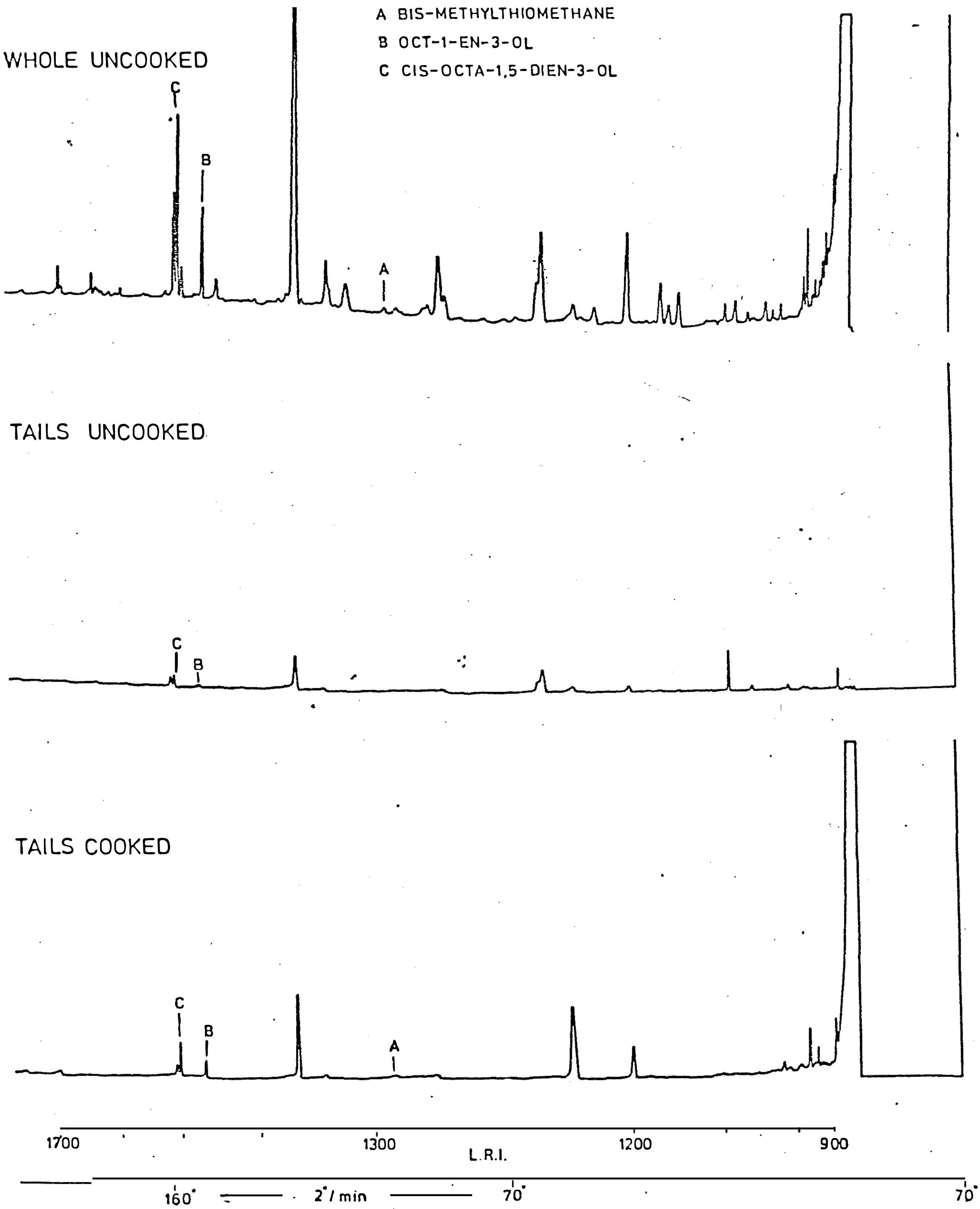
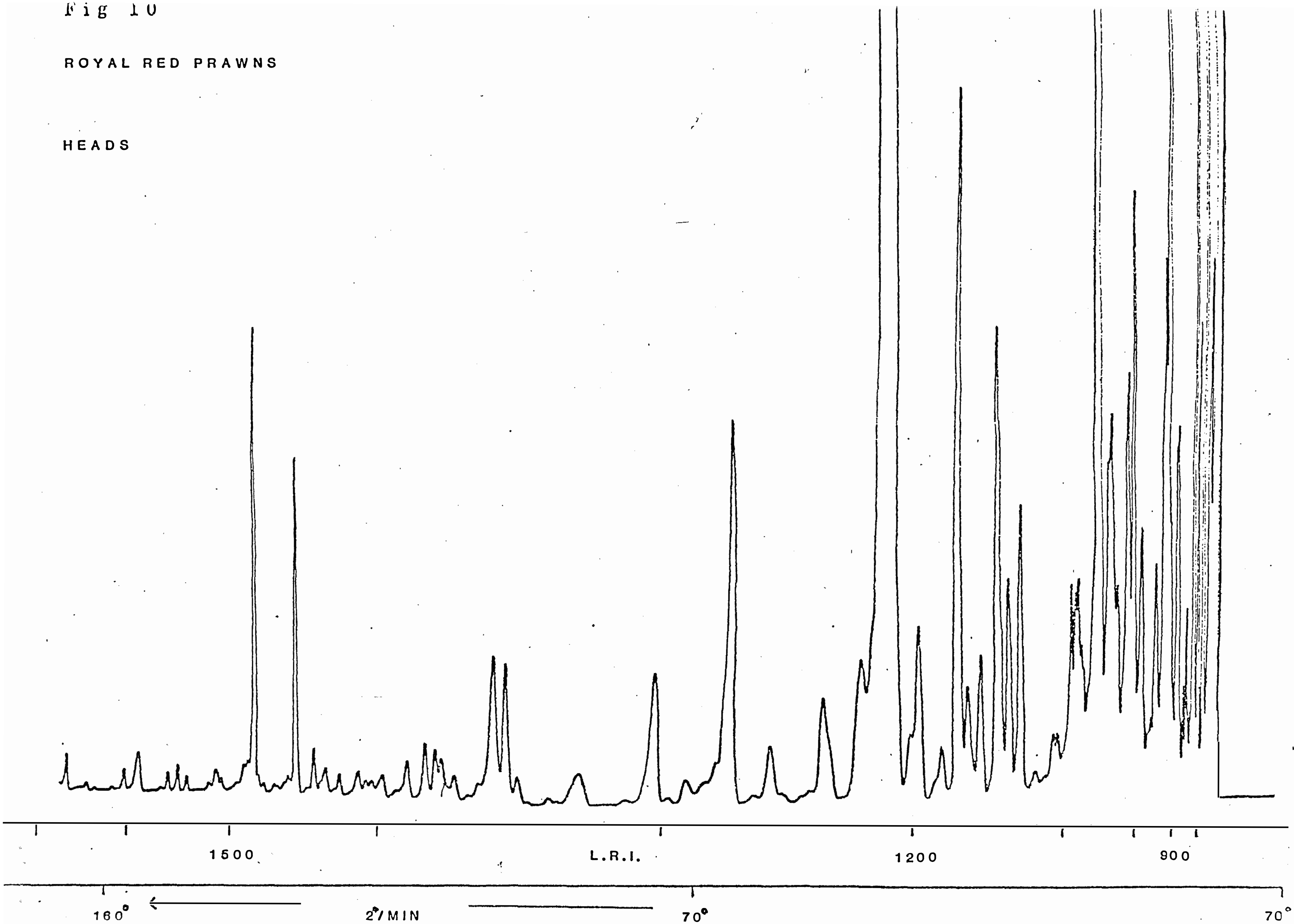
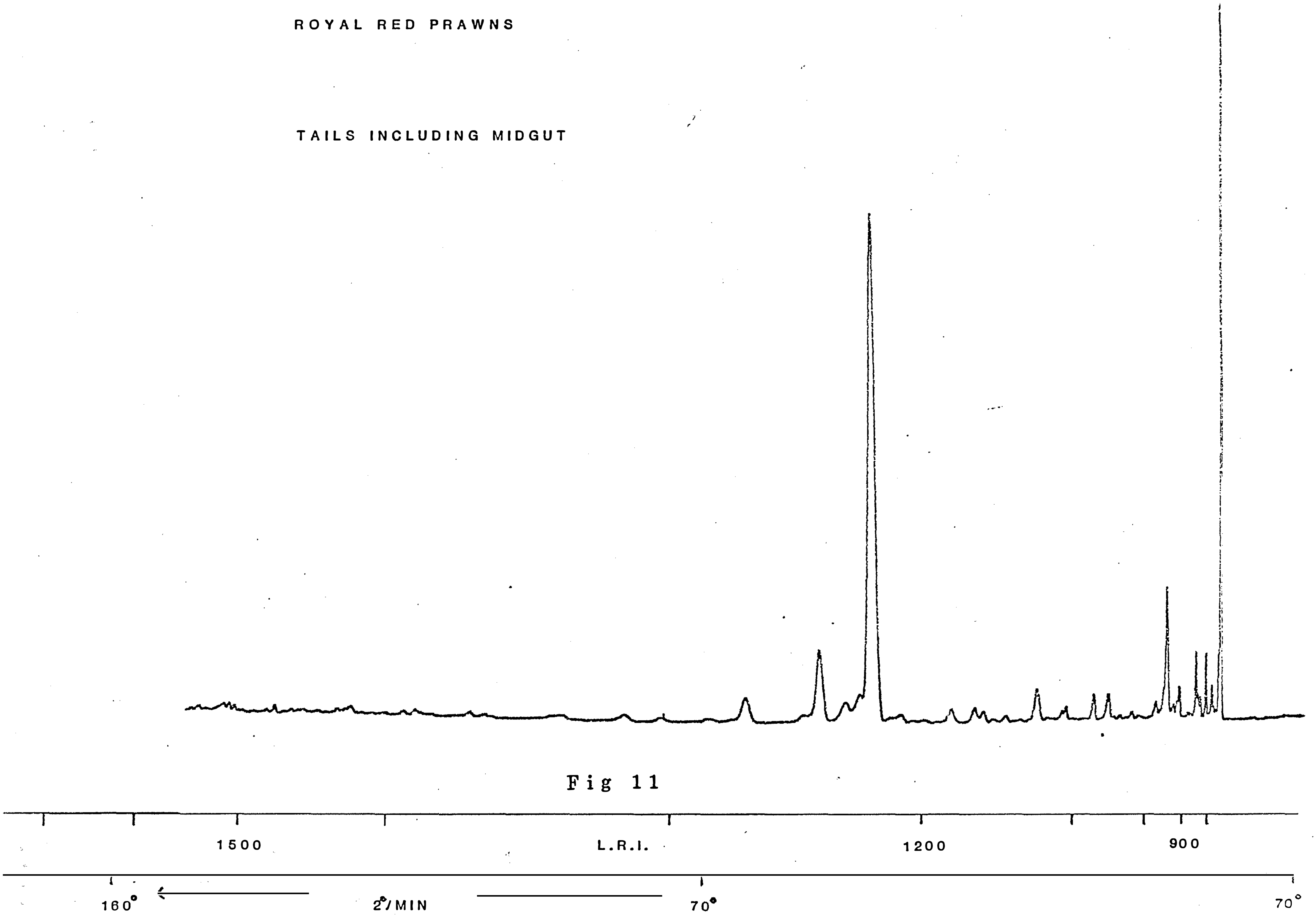


Fig 9

Fig 10





ROYAL RED PRAWNS
TAILS INCLUDING MIDGUT
1500
L.R.I.
1200
900
160°
2°/MIN
70°
70°

Fig 11

ROYAL RED PRAWNS

TAILS WITHOUT MIDGUT

Fig 12

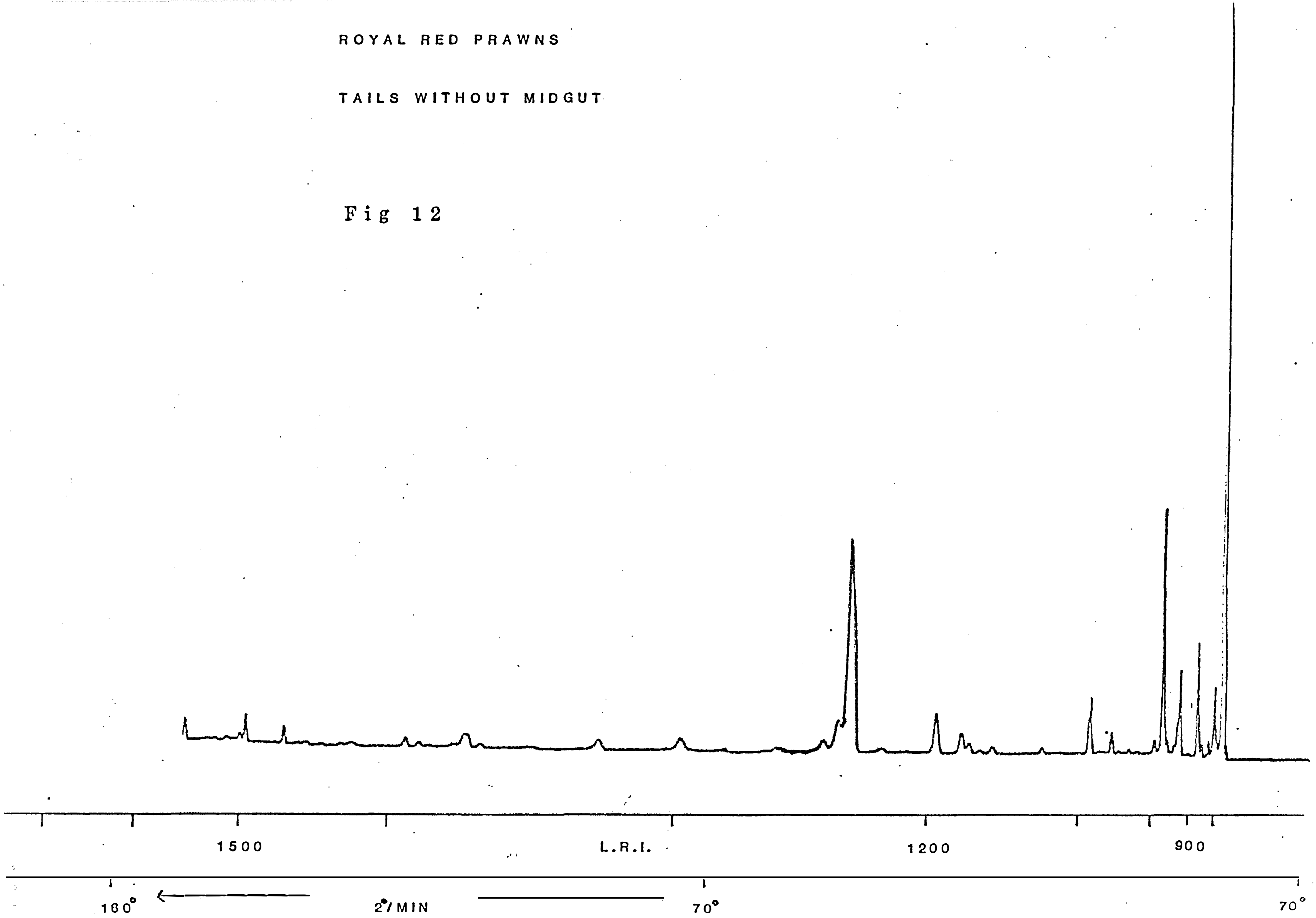


Fig 13

ROYAL RED PRAWNS

MIDGUTS