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USE OF AUTOMATED CAPILLARY COLUMN RADIO GAS CHROMATOGRAPHY IN THE IDENTIFICATION OF INSECT JUVENILE HORMONES

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SUMMARY

Automated capillary column radio gas chromatography used in conjunction with high-resolution liquid chromatography has been used for the identification of biosynthesised insect hormones and their immediate precursors. The method affords high resolution plus high sensitivity and discrimination through quantitation by liquid scintillation counting. Picogram quantities of the hormones are produced by *corpora allata* cultured *in vitro* with medium containing different ^{3}H- and ^{14}C-labelled precursors.

INTRODUCTION

The juvenile hormones, JHI (methyl 10R,11S-epoxy-7-ethyl-3,11-dimethyl-2E,6E-tridecadienoate) and JHII (methyl 10R,11S-epoxy-3,7,11-trimethyl-2E,6E-tridecadienoate), were first isolated in milligram quantities from the abdomens of the silk moth *Hyalophora cecropia*[1–3]. However, as JHs are more usually present at much lower levels (nanogram or less per insect), the rigorous identification by conventional physicochemical methods, particularly the 10R,11S configuration[4,5], is not possible. Although JHs and particularly the third homologue JHIII (methyl 10R,11-epoxy-3,7,11-trimethyl-2E,6E-dodecadienoate) have been identified in many species[1,4,6–18], there are still several important orders of insects, notably, the *Hemiptera*, whose JHs have never been identified.

Schooley[19] has recently produced a very useful review of the historical development of methods for identifying JHs on the milligram, microgram, nanogram and finally picogram scales, to which the reader is referred for a full account of the various methodological approaches, their rationales and limitations. Of the three methods which have yielded results so far, two have sensitivities in the nanogram range [gas chromatography–chemical ionisation mass spectrometry (GC–CIMS); high-resolution chromatography (capillary column GC) or high-resolution liquid chromatography (HRLC) using biosynthetically introduced radioactivity for detection], and one has sensitivities in the picogram range (two-stage derivatisation leading to the introduction of highly electrophoric groups which can be detected on electron capture

GC). GC–CIMS[18] requires expensive instrumentation, and the derivatisation is both, allegedly, time consuming[11,19] and unlikely to lead to the discovery of new JH structures, if such do exist.

In principle, it should be possible to increase greatly the sensitivity of the remaining method (monitoring of biosynthetically introduced radioactivity through high-resolution chromatography and derivatisation), by using much higher specific activity radiolabels than those employed to date.

The isolated retrocerebral glands (*corpora allata* (CA)) from all insect species so far reported on, incorporate radioactivity into the methyl ester group of JHs from S-methyl labelled methionine, during culture *in vitro*[11,12,18,20]. In some cases it is known that the radiolabel is incorporated without isotopic dilution[12,13,21,22]. Experiments reported to date have employed [^{14}C-methyl]methionine (max. specific activity 58 mCi/mmole), and yields of the radiolabelled hormone have varied greatly, depending on the species investigated, the state of activity of the glands, and the suitability of the culture medium employed[22,23]. Observed average incorporation rates ranged from *ca.* 0.02 pmol per gland pair per h (ref. 13) to *ca.* 55 pmol per gland pair per h (ref. 17), but we have failed to observe incorporation from [^{14}C-methyl]methionine by isolated retrocerebral complexes of *Calliphora erythrocephala* and *Rhodnius prolixus*[24]. As briefly reported elsewhere[25], the use of commercially available [^{3}H-methyl]methionine (specific activity 80 Ci/mmole) permits detection limits in the femtomole per gland pair per h range, so that (a) JH homologue ratios down to 1:10^{5} may be easily determined in high-activity glands and (b) the products of low-activity glands can be detected with 1000-fold greater sensitivity. However, one may foresee problems arising in the chromatographic identification of compounds containing 2 or 3 tritions per molecule, owing to isotope discrimination[26]. Moreover, Schooley *et al.*[21] chose HRLC rather than GC for their identification of JHs on the grounds that gas–liquid chromatography is more prone to generate artefacts from these thermo-labile compounds; such problems could be exacerbated by trition substitution.

Here we show that high specific activity (*ca.* 80 Ci/mmole) [^{3}H]JHs behave normally on suitable capillary gas–liquid and high-resolution liquid–solid chromatography columns. Artefacts are not generated on capillary columns, in the absence of column contamination or deterioration. Thus the inherently higher resolving power of capillary columns (compare HRLC) can be used to explore problems of geometric isomerism and diastereoisomerism in biosynthesised hormones, intermediates and hormone analogues.

EXPERIMENTAL

Radio gas chromatography (RGC)

A Pye 104 gas chromatograph was fitted with a 20M wall-coated open-tubular glass capillary column coated with Ucon HB5100 (Jaeggi, Trogen, Switzerland). An all-glass solvent-free solid injector was used (Phase Separations, Queensferry, Great Britain). The principal part of the injector is a movable glass needle onto which a solution of the sample is deposited. The carrier gas backflushes the solvent to atmosphere and the sample on the needle is injected directly onto the capillary column through the injector block at 200°. The capillary column was attached to the flame-ionisation detector (FID) with heat-shrink PTFE tubing onto 1/16-in. glass-lined steel

tube (GLT, obtained from SGE, London, Great Britain) inserted into the FID jet, eliminating, as far as possible, hot metal surfaces.

The RGC system (Figs. 1 and 2) used in this work is a simplified apparatus developed by Wels[27]. The modifications to the original design are the removal of the ^3H_2O trapping tube and the use of a single absorption fluid for the simultaneous trapping of both ^3H and ^{14}C from the FID effluent. Thus eliminating the need for two separate injections for a double-labelled sample, the PTFE effluent line from the FID was replaced with GLT, ensuring an all-glass system.

The absorption cell was as described by Wels. The absorption fluid was 5% 2-phenyl ethylamine (2-PE) (Intertechnique, Middlesex, Great Britain) in 2-ethoxy-ethanol (BDH, Poole, Great Britain). Certain batches of 2-PE obtained from other sources can give a yellow solution resulting in high background counts, typical back-

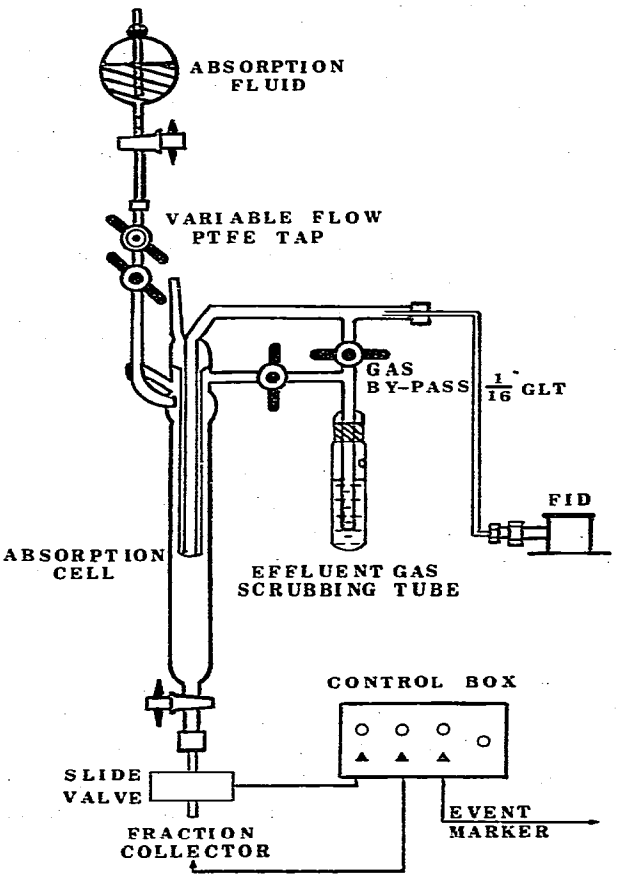

Fig. 1. Diagram of radio gas chromatography system. The absorption cell was linked to the FID outlet with 1/16-in. glass-lined tubing (SGE) with a reducing union with graphite ferrules and glass-to-metal union (SGE). The whole of the gas line was heated with 400-W heating tape (Electrothermal). Connections to the absorption fluid reservoir were with 4-mm PTFE tubing and Cheminert fittings. Fractions were made with a solenoid activated pneumatic 2-way slide valve (Chromatronix type SR1), and collected with an Isco (Model 273) fraction collector.

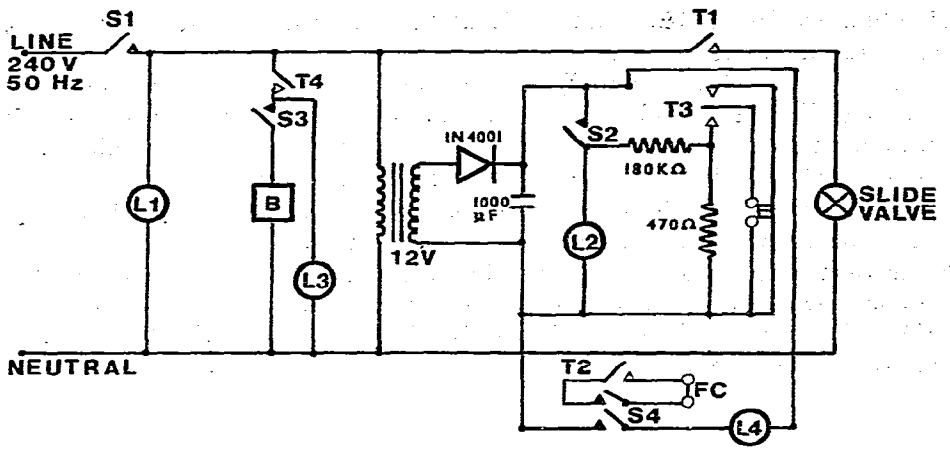

Fig. 2. Circuit diagram of the RGC control box. T1–T4 are four cam-operated microswitches of a electromechanical timer (Elremco, type D63; Electrical Remote Control). Each of the four cam positions can vary the on/off operation of the slide-valve solenoid T1, fraction collector (FC) T2, event marker pen (E) T3 and buzzer (B) T4, during the 12-sec collection cycle. S1–S4 are "enable" switches for the line voltage, event marker, buzzer and fraction collector, indicated by neon lamps L1–L4, respectively.

ground counts of 12 cpm for ^{3}H and 8 cpm for ^{14}C are obtained. Average recoveries for a double-labelled [^{14}C] [^{3}H]methyl palmitate standard were 40% for ^{3}H and 80% for ^{14}C, in close agreement with those obtained by Wels, using 10% 2-PE in 2-methoxyethanol. Although recoveries were reproducible on each particular day they varied over longer periods.

High-resolution liquid chromatography

HRLC was carried out on a Perkin-Elmer 1220 chromatograph fitted with a 5-µm Zorbax-Sil column (25 cm × 4.6 mm; DuPont, Hitchin, Great Britain), fitted with a Cecil 212 UV variable-wavelength spectrophotometer and an 8-µl flow cell. Effluent fractions were collected manually every 12 sec directly into counting vials containing 11 ml scintillant.

Liquid scintillation counting

Fractions of effluent from the absorption cell (2.0 ml) were collected automatically every 12 sec and counted in 9.0 ml of scintillation cocktail (9.8 g butyl-PBD, 100 g naphthalene, 100 ml water, in 1 l 1,4-dioxan) in a Wallac 81000 liquid scintillation spectrometer, calibrated for automatic external standardised double-label counting with standard [^{14}C]- and [^{3}H]*n*-hexadecanes (Radiochemical Centre, Amersham, Great Britain).

Materials

All solvents were AR grade and treated by passing through a mixed-bed activated silica (200°) and alumina column (BDH; "for chromatography" grade), prior to all-glass redistillation.

[1-^{14}C]- and [9,10-^{3}H]methyl palmitate were prepared by methylating the cor-

responding labelled acids (500 mCi/mmole for ^{3}H and 58 mCi/mmole for ^{14}C; Radiochemical Centre) with diazomethane and purified by thin-layer chromatography (TLC). A solution of both was diluted with unlabelled methyl palmitate to a final concentration of 100 ng per 2 μl and a ^{3}H and ^{14}C radioactivity of $1.5 \cdot 10^{4}$ dpm and $5 \cdot 10^{3}$ dpm per 2 μl, respectively.

[12-^{3}H]7-Ethyl-3,11-dimethyl-2E,6E,10Z-tridecatrienoic acid (4.3 Ci/mmole), [12-^{3}H]3,7,11-trimethyl-2E,6E-dodecatrienoic acid (3.0 Ci/mmole) (farnesenic acid), [2-^{3}H]3,7,11-trimethyl-2E,6E-dodecatrienoic acid (22 mCi/mmole) and 10,11-epoxy-6-oxa-3,7,11-trimethyl-2E,Z-tridecenoic acid (racemic) were prepared from the corresponding methyl or ethyl esters by saponification (30 h, 0.5 *N* KOH in 50% aqueous ethanol), followed by TLC. [2-^{3}H]Methyl farnesoate and ethyl epoxy-oxa-trimethyl-tridecenoate were generous gifts from Dr. A. F. White (this laboratory) and Dr. V. Jarolim (Institute of Organic Chemistry and Biochemistry, Czeckoslovak Academy of Sciences, Prague, Czeckoslovakia), respectively. [12-^{3}H]Methyl trimethyl-dodecatrienoate and [12-^{3}H]methyl ethyl-dimethyl-tridecatrienoate were obtained by sodium borotritide (*ca.* 10 Ci/mmole, Radiochemical Centre) reduction of pure samples of the 12-chloro analogues[28,29] prepared and supplied by Dr. R. C. Jennings (this laboratory) according to a novel procedure[30].

Radiochemical and isomeric purities of the acids were determined after re-esterification with ethereal diazomethane, using the quantitative RGC described herein. Products were stored under dry nitrogen at −70° in heat-treated glass vials.

Treatment of glassware

Meyer *et al.*[3] reported on the need to avoid contamination and surface reactivity of glassware used in the isolation and storage of JHs, recommending high-temperature treatment. Glassware used in the present study was washed with non-ionic detergent[31], acetone and boiling distilled water, and heated overnight at 260° or 500°[19]. Tubes for organ culture were then "coated" with polyethylene glycol (Carbowax 20M; Perkin-Elmer) from 5% aqueous solution, followed by distilled water washing and drying at 90°, to reduce adsorption of JHs onto the glassware from the culture medium[32,33].

Preparation and isolation of biosynthesised products

Isolated CA or retrocerebral complexes were obtained, and incubated in sterile organ culture medium for periods of 3–5 h, essentially as described elsewhere[34], except that the media were without non-radioactive methionine. When farnesenic acid, or analogues thereof, were added to the incubation they were incorporated into the medium without the addition of organic solvents[35]. At the end of the incubation, the glands and medium were separated[34], and the products extracted and analysed by TLC on twice washed (methanol) glass-backed silica F_{254} plates (E. Merck, Darmstadt, G.F.R.) as before[35] except that only 2 μg of authentic non-radioactive marker were added to each sample. Radioactivity on the plate was recorded either by visualisation in a beta-imager (Betagraph, Panax, Redhill, Great Britain) or by windowless gas flow-counter scanning (Berthold Scanner; 1-mm window; counting time 4 min), and the position of marker compounds determined by episcopic fluorescence-quenching densitometry (Vitatron Thin-Layer Densitometer). Samples were recovered from appropriate areas of the plate in 3–4 ml of dry diethyl ether; after

evaporation of the solvent, samples were taken up in 0.45 ml of pure iso-octane and micro-filtered through methanol-washed cellulose plugs prior to storage at −70°.

RESULTS

Isolation of biosynthesised products by TLC

Fig. 3 shows a radio thin-layer chromatogram of biosynthesised [^{3}H]JHI and olefinic esters of JHI and JHIII. It illustrates the isotope discrimination effect, which can occur in chromatographic separations, of radiolabelled JHs, at 2–3 tritions per molecule, with respect to the non-labelled mass markers. In all our experiments the biosynthesised products are recovered from the plate either by reference to the radio-scan, and not the R_F of the mass markers, or by taking a broad "JH zone" scraping.

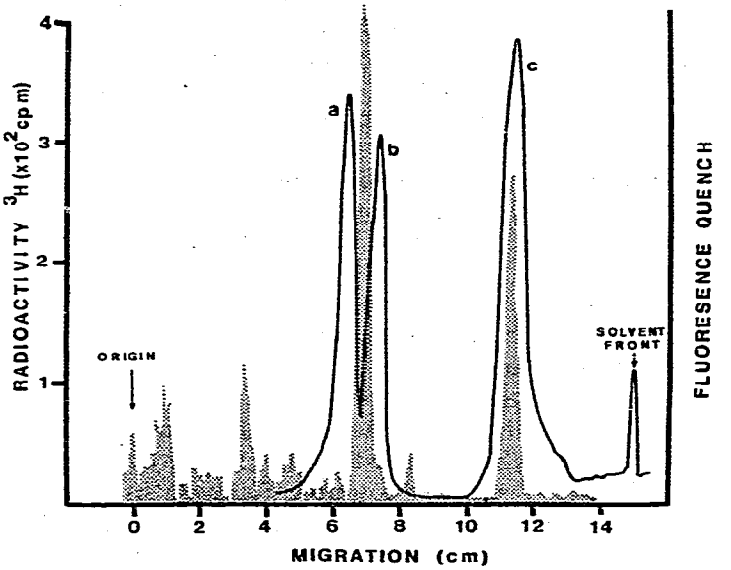

Fig. 3. Radio thin-layer chromatogram of biosynthesised products from *Periplaneta americana* CA, incubated in medium containing [12-^{3}H]3-methyl-7-ethyl-3,11-dimethyl-2E,6E,10Z-tridecatrienoic acid (4.6 Ci/mmole) and [^{3}H-methyl]methionine (80 Ci/mmole). The shaded area: radioactivity, showing non-co-chromatographic isotope effect of the biosynthesised JH1 with mass marker (b); (a) is JH III mass marker and (c) a mixture of the corresponding olefinic esters. Merck 0.25-mm silica plate was developed in S chamber using 20% ethyl acetate in xylene and iodine stained prior to fluorescence quench scanning.

RGC showing confirmation of the identity of known JHs and precursors

The identification of the biosynthesised product from *Periplaneta americana corpora allata* incubation with high specific activity [^{3}H-methyl]methionine is shown (Fig. 4) to be the 2E,6E-JHIII. This confirms previous results with non-radioactive[36] and ^{14}C-labelled[37] incubations. We have shown this particular chromatogram as it illustrates the initial stages of column deterioration, typified by the appearance of minor radioactive peaks. As column deterioration continues the magnitude of these artefacts increases, and the degradation of the main radioactive peak occurs with both high and low specific activity compounds.

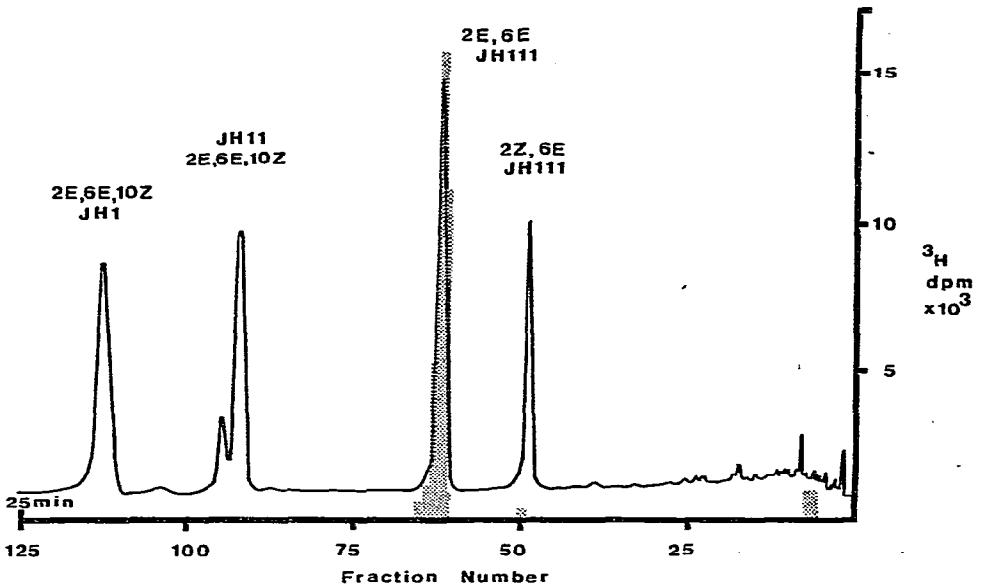

Fig. 4. Radio gas chromatogram of the "JH" zone after TLC of the products biosynthesised by *Periplaneta americana* CA incubated with [^{3}H-methyl]methionine (80 Ci/mmole). The solid line is the FID response (attenuation $1 \cdot 10^2$); the shaded histogram represents the radioactivity corresponding to 12-sec fractions of the FID pyrolysis products. Non-radioactive authentic JHs were added as internal reference markers. Conditions: 20M Ucon HB5100 capillary column at 170° with the helium carrier gas at 16 p.s.i.; chart speed, 1 cm/min.

Capillary column RGC and radio HRLC of high specific activity [^{3}H-methyl]-farnesoate biosynthesised by *P. americana* CA incubated with [2-^{3}H]farnesenic acid and [methyl-^{3}H]methionine are given in Figs. 5A and 5B, respectively. Although previously reported[15,35], these are the first chromatograms published. The use of suitable, high specific activity substrates enables the olefinic ester precursors of JHs to be readily and easily identified with capillary column RGC and this method can be applied to small amounts (1 fmole per pair) of methyl farnesoate or its homologues occurring spontaneously in glands from other species[38].

Identification of new structures isolated from CA incubation medium. The ability of *Periplaneta americana* glands to utilise novel analogue substrates is shown in Fig. 6. In this experiment, the glands were incubated with an analogue of farnesenic acid, consisting of varying proportions of all possible optical and double bond isomers. The structural constraints of the O-methyl transferase in the biosynthetic pathway are illustrated by the complete failure to incorporate radioactivity into the 2Z isomers of epoxy-oxa-trimethyl tridecenoate. Note that three of the four diastereoisomer pairs of the 2E isomer have been separated, and that the radioactivity is incorporated in approximately the same ratio as the original isomeric composition.

Specific activity determinations. Specific activity estimations of ^{3}H-labelled JH, JH analogues, precursors were conveniently carried out by RGC. For example, 0.6 mg of [12-^{3}H]methyl-3,7,11-trimethyl-2E,6E,10Z-tridecatrienoate were synthesised (9.4 mCi). Accurate specific radioactivity values were obtained by co-injection of a very small aliquot of the sample with an internal standard of 40.0 ng [^{3}H]methyl

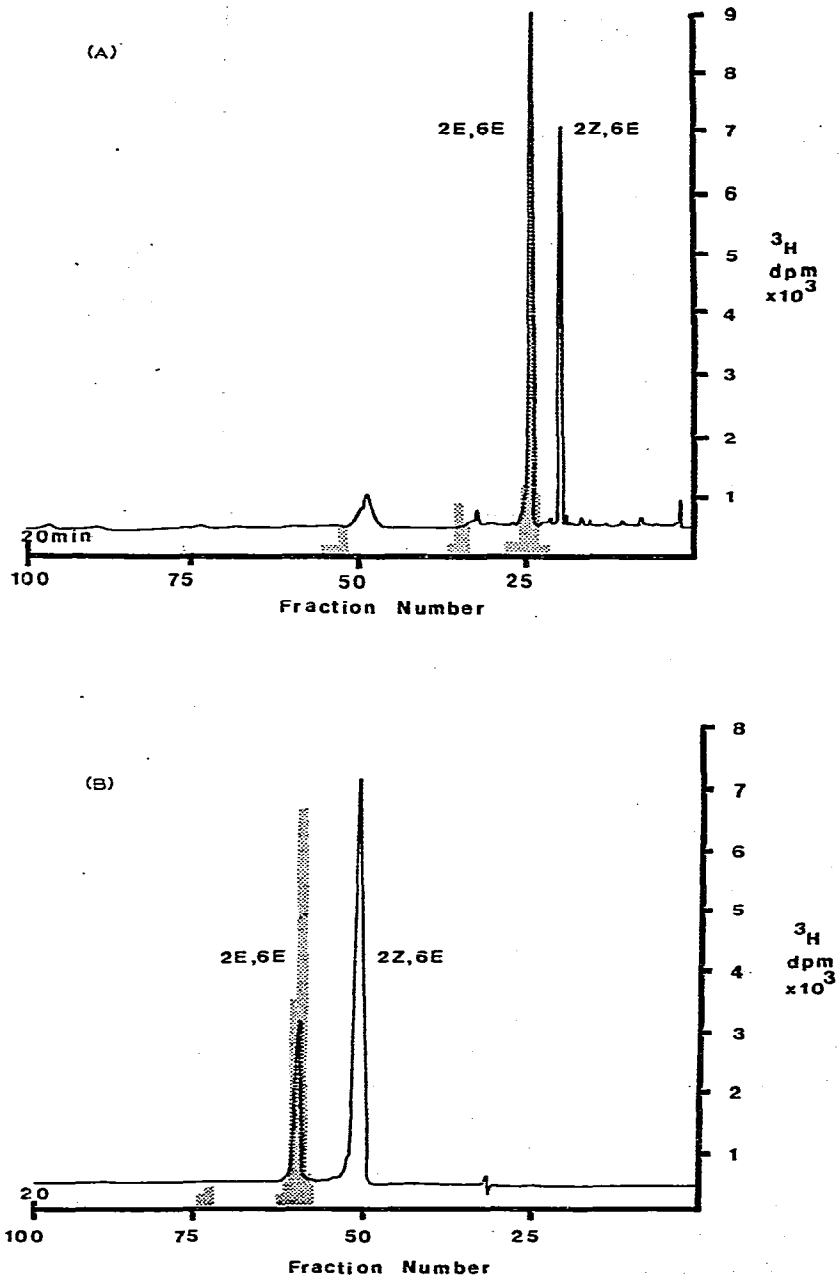

Fig. 5. A and B are RGC and radio HRLC, respectively, of the methyl farnesoate (MF) zone from the TLC of the products of *Periplaneta americana* CA, stimulated with farnesenic acid, and cultured with [^{3}H-methyl]methionine (80 Ci/mmole). Non-radioactive geometric isomers of MF were added as internal reference markers. RGC conditions: 20M Ucon HB5100 capillary column at 170° with the helium carrier gas at 16 p.s.i.; chart speed, 1 cm/min. Radio HRLC conditions: 25 cm × 4.6 mm 5-μm Zorbax-Sil column; 6% ether in *n*-pentane (50% water-saturated) at 0.5 ml/min; UV detection at 225 nm and 0.2 a.u.f.s.

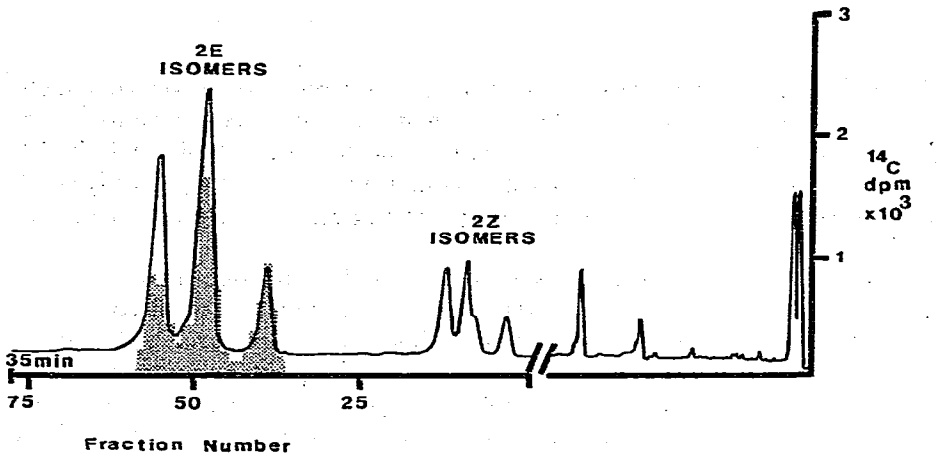

Fig. 6. RGC of the products biosynthesised by *Periplaneta americana* CA cultured in medium containing 10,11-epoxy-6-oxa-3,7,11-trimethyl-2-E,Z-tridecenoic acid and [^{14}C-methyl]methionine (48 mCi/mmole). Non-radioactive authentic methyl ester added as internal reference marker. Conditions: 20M Ucon HB5100 capillary column at 155° with helium carrier gas at 8 p.s.i.; chart speed, 1 cm/min.

palmitate ($1.03 \cdot 10^4$ dpm). The internal standard was used to quantify both the mass peak of the trienoate ester, by triangulation, and the efficiency of ^3H_2O trapping in the absorption cell (62% in this particular case). Calculation showed that 93 ng of trienoate had been injected containing $3.4 \cdot 10^6$ dpm, affording a specific activity of 4.6 Ci/mmole.

Radioactivity recoveries of [12-^{3}H]JHIII

The recovery of a labelled JH from the capillary column RGC was tested using a synthetic [12-^{3}H]JHIII sample, which was radiochemically pure by TLC. Approximately 100 ng of a Z, E; E, Z and E, E isomeric mixture was co-injected with a 50 ng [^{3}H]methyl palmitate internal standard. Integration of the mass peaks (Model CRS304 integrator, Infotronics) showed an isomer composition of 4.3% 2Z, 6E, 3.6% 2E, 6Z and 91.5% 2E, 6E; the corresponding distribution of radioactivity was 5.4%, 4.3% and 90.3%. After correction for the ^3H_2O trapping efficiency (71% in these experiments) the total recovery of the radiolabelled JHIII was found to be 97%, showing negligible losses or degradation during chromatography.

Degradation of the column caused by unclean carrier gas or accumulation of extraneous material at the injection point, can affect recoveries of both high and low specific activity compounds. The effects upon radioactive recovery, and production of artefactual peaks, can be far more pronounced than the effects on column performance as shown by the mass tracing. Generally, column degradation is slowed down by (a) prior purification of sample and solvents and (b) purification of the carrier gas with an activated molecular sieve. Column life can be extended somewhat, by removing the first loop of the column when sample loss or breakdown is first observed.

DISCUSSION

We have shown that high-resolution RGC is a simple and satisfactory technique for the analysis of biosynthesised JHs and their analogues. Here, we have employed highly active glands from *Periplaneta americana* to biosynthesise JHs in 10-μCi range, from high specific activity L-[^{3}H-methyl]methionine, *in vitro*. As only small aliquots are required for analysis, the majority of the sample can be used for further biological studies.

In our laboratory, we have already used the method to identify or confirm the identification of, low specific activity JHs (mostly ^{14}C-labelled) released by cultured *corpora allata* from *Periplaneta americana*[37], *Schistocerca gregaria*[15], *Diploptera punctata*[17], *Locusta migratoria* and *Tenebrio molitor*[38]. The spontaneous rates of JH biosynthesis were sufficiently high (1–50 pmoles per gland pair per h) in all these cases, to permit ready identification of the principle hormone at low specific activity (30–58 mCi/mmole). However, analyses of the JH content of the insect *Nauphoeta cinerea*, show the importance of high-sensitivity detection systems. Trautmann *et al.*[8] discovered only JHIII in extracts analysed by capillary GC–electron impact MS (GC–EIMS), whereas Lanzrein *et al.*[9] used the more sensitive CIMS to show the presence of smaller, but possibly highly significant, quantities of the higher homologues. The present study shows that biosynthesised JHs containing 2–3 tritions per molecule are compatible with capillary column RGC, enabling this technique to be used to determine the ratios of JH homologues biosynthesised by the glands *in vitro* with high sensitivity. Thus, for example, glands from *Periplaneta americana* synthesise exclusively JHIII under these conditions, since JHII and JHI would have been detectable at the 0.01 % level.

Our failure to observe significant isotope discrimination with JHs and JH-analogues tritium-labelled at 70–80 Ci/mmole, during our particular high-resolution GC and LC separations, should not be assumed to apply for alternative separation systems. The separation of multiple-position ^{3}H-labelled from non-radiolabelled or single-position ^{14}C-labelled hormones on TLC (Fig. 3) means that caution must be used in identifying materials by TLC, particularly if the material must be recovered without a change in specific activity or mixed isotope ratio. If, for reasons beyond experimental control, the level of radioactivity in a sample is too low for gas-flow detection *in situ*, one must resort to the taking of a wide "cut" from the plate, bearing in mind that the radio-labelled products have slightly lower R_F values than non-radioactive authentic markers. With this proviso, high specific activity [^{3}H-methyl]methionine incorporations followed by preparative TLC and then high-resolution capillary RGC, should prove to be a valuable method for the identification of JHs produced by *corpora allata* from insect spp. having very low activity glands. Similarly, the yields of radio-labelled methyl farnesoate (or its higher homologues) from cultures of whole glands *in vitro* is usually very much less than the yield of JHs, since it is a steady-state intermediate[22] and does not accumulate under conditions of spontaneous synthesis[15]; the higher sensitivity afforded by the present method should facilitate its identification and quantitation in low activity glands such as those from *Leptinotarsa decemlineata*[20] and *Tenebrio molitor*[23].

Capillary GC has a higher resolving power than currently available HRLC systems particularly in respect of geometrical isomerism of known JHs: all isomers

are fully resolved, and all natural hormones possess the 2E, 6E or 2E, 6E, 10Z configuration. The last two enzymes in the biosynthetic pathway, the O-methyl transferase and 10,11-epoxidase, appear to have low homologue specificity, as can be shown by the efficient utilisation of unnatural (for the particular spp. and stage of glands used) side-chain homologues of farnesenic acid[25,35]. Capillary RGC should permit a detailed investigation of the accompanying isomeric specificity (or otherwise) of these enzymes. Here we have shown that the O-methyl transferase in glands from *Periplaneta americana* does not methylate the unnatural 2Z isomers of a farnesenic acid analogue, suggesting important structural constraints close to the reactive site of the enzyme. The analogue employed for this study (racemic 10,11-epoxy-6-oxa-3,7,11-trimethyl-2E,Z-tridecenoic acid) was relatively flexible, with only two rotational restrictions (2,3 double bond and 10,11 epoxide ring), but contained three optically active centres (four diastereomeric pairs). Here we have found that the enzyme utilises all four diastereomers approximately in proportion to their concentration in the incubation medium, suggesting some degrees of freedom in that part of the farnesenic acid binding domain removed from the reactive centre (carboxylic acid group). It remains to be established whether the two enantiomers of each diastereomeric pair are equally active substrates. Clearly, capillary RGC can be used to investigate some stereochemical problems relating to JH biosynthesis, but HRLC may be the system of choice for others. For example, the chirality of the epoxide ring in JHs can be determined by separation of the diastereoisomeric esters derived from the corresponding diols and an optically active acid[12–14]. Although simple esters of the convenient acid employed, (+)-1-methoxy-1-trifluoromethylphenylacetic acid, can be analysed by GC[39], problems of thermal instability with compounds in the JH series can be anticipated. The reliability of the structural determination of an unknown JH is greatly increased by establishing co-chromatography on high-resolution systems with authentic compounds, of both the biosynthesised material and its product from a reasonably specific chemical derivatisation[19]. The formation of "methoxyhydrins", by acid methanol attack on the epoxide group, is particularly suitable for JHs since the reaction proceeds smoothly to completion on the picogram scale[21,24]. We shall show elsewhere[38] that the methoxyhydrins of JHs are readily analysed on the capillary RGC system employed here.

RGC has also proved invaluable in determining the specific radioactivity of the synthetic intermediate farnesenic acid and its analogues. Regrettably these radiolabelled intermediates cannot be obtained commercially and are only available in our laboratory in milligram and sub-milligram quantities, so that gravimetric determination of the specific activities is unsatisfactory.

The inherently higher FID sensitivity of capillary column GC rather than packed column GC has enabled us to perform accurate specific activity measurements on these precursors (usually as their methyl esters) at activities of up to 6 Ci/mmole. These precursors can be employed in double-labelled biological experiments, to determine the efficiency of incorporation into JHs from [^{14}C-methyl]methionine on subnanogram quantities of material[15,35]. The more direct method of determining $^{14}C/^{12}C$ ratios in the hormone by GC–EIMS[12] requires microgram quantities of material (thus precluding its routine experimental use), and might lead to marked underestimation of the percent utilisation of methionine in the case of low activity glands if significant amounts of non-radiobiosynthesised JH are present in the tissue (*cf.*

ref. 14). Information on the stoichiometry of exogenous labelled-methionine incorporation by *corpora allata* into natural JHs is now widely required to validate the use of this incorporation to determine the physiological level of synthetic activity in isolated glands. This information has not always been available[11,17,20].

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REFERENCES

1 H. Röller, K. H. Dahm, C. C. Sweeley and B. M. Trost, *Angew. Chem., Int. Ed. Engl.*, 6 (1967) 179.
2 A.S. Meyer, H. A. Schneiderman, E. Hanzmann and J. H. Ko, *Proc. Nat. Acad. Sci. U.S.*, 60 (1968) 853.
3 A. S. Meyer, E. Hanzmann, H. A. Schneiderman, L. I. Gilbert and M. Boyette, *Arch. Biochem. Biophys.*, 137 (1970) 190.
4 P. D. Shirk, K. H. Dahm and H. Röller, *Z. Naturforsch. C*, 31 (1976) 199.
5 A. S. Meyer, *Mitt. Schweiz. Ent. Gesellschaft*, 44 (1971) 37.
6 K. H. Dahm and H. Röller, *Life Sciences*, 9 (1970) 1397.
7 K. H. Trautmann, A. Schuler, M. Suchý and H.-K. Wipf, *Z. Naturforsch. C*, 29 (1974) 161.
8 K. H. Trautmann, P. Masner, A. Schuler, M. Suchý and H.-K. Wipf, *Z. Naturforsch. C*, 29 (1974) 757.
9 B. Lanzrein, M. Hashimoto, V. Parmakovich, K. Nakanishi, R. Wilhelm and M. Lüscher, *Life Sciences*, 16 (1975) 1271.
10 L. W. van Broekhoven, A. C. van der Kerk-van Hoof and C. A. Salemink, *Z. Naturforsch. C*, 30 (1975) 726.
11 K. H. Dahm, G. Bhaskaran, M. G. Peter, P. D. Shirk, K. R. Seshan and H. Röller, in L. I. Gilbert (Editor), *The Juvenile Hormones*, Plenum Press, New York, 1976, pp. 19–47.
12 K. J. Judy, D. A. Schooley, L. L. Dunham, M. S. Hall, B. J. Bergot and J. B. Siddall, *Proc. Nat. Acad. Sci. U.S.*, 70 (1973) 1509.
13 K. J. Judy, D. A. Schooley, M. S. Hall, B. J. Bergot and J. B. Siddall, *Life Sciences*, 13 (1973) 1511.
14 K. J. Judy, D. A. Schooley, R. G. Troetschler, R. C. Jennings, B. J. Bergot and M. S. Hall, *Life Sciences*, 16 (1975) 1059.
15 G. E. Pratt, S. S. Tobe and R. J. Weaver, *Experientia*, 30 (1975) 120.
16 R. C. Jennings, K. J. Judy, D. A. Schooley, M. S. Hall and J. B. Siddall, *Life Sciences*, 16 (1975) 1033.
17 S. S. Tobe and B. Stay, *Gen. Comp. Endocrinol.*, 31 (1977) 138.
18 B. Lanzrein, V. Gentinetta and M. Lüscher, in H. H. W. Weltruiz and J. T. Wiebes (Editors), *8th Congr. Int. Union Study Social Insects, Wageningen, The Netherlands, 1977*, Centre for Agricultural Publishing and Documentation, Wageningen, 1977, pp. 265–268.
19 D. A. Schooley, in R. B. Turner (Editor), *Analytical Biochemistry of Insects*, Elsevier, Amsterdam, 1977, pp. 241–287.
20 S. J. Kramer, *Ph.D. Thesis*, Wageningen Agricultural University, Wageningen, 1978.
21 D. A. Schooley, K. J. Judy, B. J. Bergot, M. S. Hall and R. C. Jennings, in L. I. Gilbert (Editor), *The Juvenile Hormones*, Plenum Press, New York, 1976, pp. 101–117.
22 S. S. Tobe and G. E. Pratt, *Biochem. J.*, 144 (1974) 107.
23 R. J. Weaver, G. E. Pratt and A. F. Hamnett, *Gen. Comp. Endocrinol.*, 34 (1978) 1.
24 G. E. Pratt and A. F. Hamnett, unpublished results.
25 G. E. Pratt, A. F. Hamnett, J. R. Finney and R. J. Weaver, *Gen. Comp. Endocrinol.*, 34 (1978) 63.

26 E. A. Evans, *Tritium and Its Compounds*, Butterworths, London, 1966.
27 C. M. Wels, *J. Chromatogr.*, 142 (1977) 459.
28 W. S. Johnson, T. J. Brockson, P. Loew, D. H. Rich, L. Werthemann, R. A. Arnold, T. Li and D. J. Faulkner, *J. Amer. Chem. Soc.*, 92 (1970) 4463.
29 H. Rode-Gowal, S. Abbott, D. Meyer, H. Röller and K. H. Dahm, *Z. Naturforsch. C*, 30 (1975) 392.
30 R. C. Jennings, unpublished results.
31 G. E. Pratt, *Insect Biochem.*, 5 (1976) 595.
32 B. D. Hammock, S. S. Gill, V. Stamoudis and L. I. Gilbert, *Comp. Biochem. Physiol.*, 53B (1976) 263.
33 Ch. Giese, K. D. Spindler and H. Emmerich, *Z. Naturforsch. C*, 32 (1977) 158.
34 R. J. Weaver and G. E. Pratt, *Physiol. Entomol.*, 2 (1977) 59.
35 G. E. Pratt and S. S. Tobe, *Life Sciences*, 14 (1974) 575.
36 P. J. Müller, P. Masner, K. H. Trautmann, M. Suchý and H.-K. Wipf, *Life Sciences*, 15 (1974) 915.
37 G. E. Pratt, R. J. Weaver and A. F. Hamnett, in L. I. Gilbert (Editor), *The Juvenile Hormones*, Plenum Press, New York, 1976, pp. 164–178.
38 R. J. Weaver, G. E. Pratt, A. F. Hamnett and R. C. Jennings, in preparation.
39 J. A. Dale, D. L. Dull and H. S. Mosher, *J. Org. Chem.*, 34 (1969) 2543.