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# Biogenic amines: their occurrence, biosynthesis and metabolism in the locust, *Schistocerca gregaria*, by gas chromatography–negative-ion chemical ionisation mass spectrometry

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## ABSTRACT

Extraction–derivatisation techniques have been developed for the unambiguous identification of biogenic amines, and their putative amino acid precursors and metabolites (both major and minor), in single ventral thoracic nerve cords of the locust, *Schistocerca gregaria*, by the use of gas chromatography–negative-ion chemical ionisation mass spectrometry with selected ion monitoring. In addition the configuration of that enantiomer of *p*-octopamine present in the thoracid nervous system of the locust was established as *R* using the chiral derivatisation reagent, (−)-heptafluorobutyrylphenylalanyl chloride.

## INTRODUCTION

Considerable confusion exists concerning the proposed biosynthesis, identification, quantitation and metabolism of biogenic amines in insects. Undoubtedly, major factors in that confusion have been lack of sensitivity and specificity of the methods used.

Early attempts to identify free amino acids in the nervous system of the cockroach, *Periplaneta americana*, involved the use of non-specific radiolabelled dansyl derivatives [1] and the use of ion-exchange chromatography followed by colorimetric assay [2]. More recently, high-performance liquid chromatography (HPLC) has been used to report the presence of 3,4-dihydroxyphenylalanine (DOPA) in larval blowflies [3] and nematode parasites [4]. Phenylalanine and tyrosine have been determined in human plasma by gas chromatography–negative-ion chemical ionisation mass spectrometry (GC–NICIMS) using pentafluorobenzyl-trifluoroacetyl derivatives [5]. However, a new method reported [6] for the determination of L-DOPA in plasma and cerebrospinal fluid, which combines

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N,O-acetylation of amino acids in aqueous media, preparation of pentafluorobenzyl esters under anhydrous conditions and subsequent analysis by GC–NICIMS, failed to work in our hands.

It has been proposed that *p*-octopamine, dopamine and 5-hydroxytryptamine (5-HT) are neurotransmitters in the central nervous system of insects [7–10]. In our earlier work, we established unequivocally by GC–NICIMS that the principal amines in cockroach brain [11] and locust thoracic nerve cords [12] are *p*-tyramine, *p*-octopamine, 5-HT and noradrenaline. These compounds and the pathways involved in their biosynthesis and metabolism are of considerable interest *per se* and also in relation to the systematic development of insecticides.

Various mechanisms have been studied in relation to the inactivation of monoamines in insects which include N-acetylation [13–16], oxidative deamination [17,18], O-sulphate or ß-alanyl conjugation [19,20] and sodium-sensitive and sodium-insensitive uptake mechanisms [21]. The majority of evidence accumulated favours N-acetylation as the principal mechanism of inactivation in these species [22].

Various sensitive, albeit non-specific, methods are available for the detection of N-acetylmetabolites: from thin-layer chromatography (TLC) [13] to an enzymatic radiochemical assay [23] to the more elegant method of HPLC with electrochemical detection (ED) [24].

The enantiomeric composition of drugs will become increasingly important as more studies reveal that, whereas one enantiomer of a chiral molecule may have the desired therapeutic activity, the other may be inactive, antagonistic, toxic or have completely different pharmacological properties [25,26]. The enantiomeric composition of endogenous substances (*e.g.* neurotransmitters) is equally important with respect to their activity at receptors in the nervous system.

Enantiomers of chiral molecules have the same chromatographic properties, but chromatography may be used to analyse enantiomeric mixtures if either a chiral stationary (or mobile) phase is used or if diastereomeric derivatives are formed *via* reaction with an enantiomerically pure reagent.

GC–MS (which cannot be regarded as a routine technique) has the advantage over other methodologies in that compounds may be identified with a high degree of specificity by high-resolution capillary GC combined with the monitoring of characteristic ions in the mass spectrum of a compound. This technique can afford characteristic ratios of ion intensities or the $m/z$ value of a particular ion may be changed in a predictable manner to provide additional proof of identity. This may be achieved by the preparation of a different derivative of the same chemical class.

## EXPERIMENTAL

### *Gas chromatography–mass spectrometry*

GC–MS in the NICI mode was carried out using a Hewlett-Packard 5988A gas chromatograph–mass spectrometer interfaced with an HP RTE-6/VM data system. The following MS conditions were used: the instrument was tuned in the NICI mode to the ions at *m/z* 452, 595 and 633 from the perfluorotributylamine (PFTBA) calibrant, source temperature was 140°C (100°C for the analysis of the N-acetyl metabolites), electron energy 200 eV and methane reagent gas was introduced to give a source pressure *ca.* 0.9 Torr. The gas chromatograph was fitted with an SGE BP1 (12 m × 0.2 mm I.D., 0.25 μm film thickness) capillary column and helium carrier gas was used with a head pressure of 0.35 bar.

### *Materials and reagents*

All solvents used in extraction were HPLC-grade (Rathburn Chemicals, Walkerburn, U.K.). Chemicals were purchased from Aldrich (Gillingham, U.K.), Sigma (Poole, U.K.) or Fluorochem (Glossop, U.K.).

Previously established procedures were used for the synthesis of deuteriated internal standards and standards: [2,3,4,5,6-$^2H_5$]phenylalanine, [3,5-$^2H_2$]tyrosine and [2,5,6-$^2H_3$]-DOPA [27,28]; [3,5-$^2H_3$]*p*-tyramine deuteriochloride, [α,α,β,3,5-$^2H_5$]*p*-octopamine hydrochloride and [2,6-$^2H_3$]dopamine deuteriochloride [11]; N-acetyl-*p*-octopamine, N-acetyl-*p*-tyramine, [$^2H_3$]N-acetyl-*p*-octopamine, [$^2H_3$]-N-acetyl-*p*-tyramine and [$^2H_3$]-N-acetyldopamine [29] and [$^2H_3$]-*p*-hydroxyphenylacetic acid; [$^2H_5$]3,4-dihydroxyphenylacetic acid, [$^2H_5$]homovanillic acid and [$^2H_3$]-*p*-hydroxymandelic acid [30]. *o*-Octopamine, *o*-synephrine and the enantiomers of *m*- and *p*-octopamine were available from our previous work [31]. 4,6-Difluoro-5-hydroxytryptamine was kindly donated by Dr. K. L. Kirk (NIH, Bethesda, MD, U.S.A.).

### *Extraction and derivatisation*

Freshly dissected ventral thoracic nerve cords from two to seven days old adult locusts, *Schistocerca gregaria*, were transported overnight from the University of Cambridge in solid $CO_2$ and stored at −20°C prior to analysis. Prolonged storage did not appear to have any adverse effect on the concentrations of amino acids, amines or N-acetyl metabolites in the tissues. All the tissues were obtained from crowded laboratory cultures of animals fed on wheat seedlings.

*Amino acids.* These were extracted and analysed as described previously [32]. Briefly: single ventral thoracic nerve cords were homogenised manually in a ground-glass homogeniser with HCl (0.1 *M*, 0.5 ml). The homogenate was centrifuged and the HCl was eliminated from the supernatant by azeotropic evaporation with acetonitrile under a stream of nitrogen. The amino acids in the residue were reacted either with hexafluoroisopropanol (HFIP) and pentafluoropropionic anhydride (PFPA) or with trifluoroethanol (TFE) and PFPA. The

derivatives were then analysed by GC–NICIMS (GC conditions being as described previously [32]) and quantified against the deuteriated internal standards.

*Phenolamines and catecholamines.* Extraction and derivatisation was carried out as described previously [33]. Briefly: single ventral thoracic nerve cords were homogenised. The supernatant was added to aqueous potassium phosphate buffer (1 *M*, pH 7.2, 1 ml), reacted with 3,5-ditrifluoromethylbenzoyl chloride (DTFMBCl) and extracted with ethyl acetate. The extracts were shaken with aqueous ammonium hydroxide, the extract was dried, the solvent removed and hydroxyl groups were trimethylsilylated by reaction of the residue with bistrimethylsilylacetamide (BSA). Alternatively, in order to form *tert.*-butyldimethylsilyl (TBDMS) derivatives, the residue was reacted with TBDMS chloride (TBDMSCl) and imidazole in dimethylformamide. The derivatised extracts were then analysed by GC–NICIMS; the GC conditions were identical to those described for the analysis of amino acids.

*N-Acetylated metabolites.* These were analysed as described previously [29]. Briefly: single ventral thoracic nerve cords were homogenised manually in a ground-glass homogeniser with acetonitrile. The homogenate was centrifuged as before and the supernatant was evaporated under a stream of nitrogen. The residue was derivatised with either PFPA or trifluoroacetic anhydride (TFAA) and then analysed by GC–NICIMS. The GC conditions used were as described previously.

*Acidic metabolites.* Single ventral thoracic nerve cords were homogenised and centrifuged as described above for amino acids. The supernatant was added to aqueous potassium phosphate buffer (1 *M*, pH 7.2, 1 ml), acetic anhydride (30 $\mu$l) was added and the aqueous reaction mixture vortexed vigorously (1 min) on a mechanical shaker. The mixture was acidified with HCl (2 *M*, 0.5 ml) and the resultant derivatised compound extracted with ethyl acetate. The combined extracts were dried and the ethyl acetate was removed by evaporation with a stream of nitrogen.

Derivatisation was carried out as described previously [30]. The residue was dissolved in acetonitrile and reacted (10 min, room temperature) with 3,5-ditrifluoromethylbenzyl bromide (DTFMBBr) and triethylamine. Ethyl acetate and then hexane were added and the reaction mixture was allowed to stand for 10 min at room temperature. The resultant solution was shaken with HCl (2 *M*, 1 ml) until the organic layer became clear. It was separated, dried and the ethyl acetate was removed by evaporation with a stream of nitrogen. The dried residue was reacted (10 min, 65°C) with pyridine and acetic anhydride to form acetylated DTFMBBr (Ac-DTFMBBr) derivatives and excess reagent was removed under a stream of nitrogen. The residue thus obtained was dissolved in ethyl acetate prior to analysis by GC–NICIMS. The GC conditions were as follows: injector temperature, 250°C; transfer line temperature, 280°C; the oven temperature was maintained at 140°C for 1 min, then programmed at 10°C $min^{-1}$ to 300°C.

*5-HT and N-acetylhydroxytryptamine (NA-HT).* 5-HT and NA-HT were determined using a previously published GC–NICIMS technique [34].

*Stereochemical analysis of p-octopamine.* The chiral derivatising reagent, (−)-heptafluorobutyrylphenylalanyl chloride [(−)-HFBPALCl], was prepared in our laboratories from L-(−)-phenylalanine as follows: L-(−)-phenylalanine (1 g) was stirred with heptafluorobutyryl chloride (HFBCl) (2 g) in acetonitrile (50 ml) at room temperature for 4 h. The solvent and excess HFBCl were removed by rotary evaporation; a portion (100 mg) of the product was dissolved in thionyl chloride (2 ml) and the solution heated for 1 h at 60°C. The excess thionyl chloride was evaporated under a stream of nitrogen and the residue dissolved in ethyl acetate (2 ml). This solution was prepared freshly before each experiment by reaction of HFB-phenylalanine with thionyl chloride.

(−)-*m*-Octopamine (20 ng) was added as an internal standard to a single ventral thoracic nerve cord which was then homogenised with HCl (0.1 *M*, 0.5 ml) and the suspension was centrifuged. The supernatant was mixed with aqueous potassium phosphate buffer (1 *M*, pH 7.2, 1 ml), (−)-HFBPALCI (50 μl, 100 mg/ml in ethyl acetate) was added and the solution shaken vigorously for 10 min. Ethyl acetate (2 ml) was then added; the upper organic layer was removed and treated as described for the preparation of DTFMB–TBDMS derivatives of biogenic amines [32]. The resultant (−)-HFBPAL–TBDMS derivatives were analysed by GC–NICIMS. The gas chromatograph was programmed as follows: 100°C for 1 min, then 30°C $min^{-1}$ to 240°C and finally 1°C $min^{-1}$ to 260°C.

## RESULTS AND DISCUSSION

The mass spectra for HFIP–pentafluoropropionyl (PFP) derivatives of amino acid precursors of biogenic amines are summarised in Table I. The ion current in the mass spectra was generally carried by four or five large ions. Under NICI conditions the mass spectrum of the HFIP–PFP derivative of phenylalanine contained four principal fragment ions. A typical loss from the molecular ion was 28

TABLE I

PRINCIPAL IONS IN THE MASS SPECTRA OF HFIP–PFP AND TFE–PFP DERIVATIVES OF PHENYLALANINE, TYROSINE AND DOPA

| Compound | *I* value | Base peak | Other significant ions |
|---|---|---|---|
| *HFIP-PFP* | | | |
| Phenylalanine | 1091 | 433 | 298 (76) |
| Tyrosine | 1301 | 475 | 427 (94) |
| DOPA | 1386 | 765 | 638 (44) |
| *TFE-PFP* | | | |
| Phenylalanine | 1444 | 373 | 230 (59) |
| Tyrosine | 1518 | 407 | 301 (28) |
| DOPA | 1548 | 569 | 697 (21) |

a.m.u., due to loss of CO; this interpretation of the loss was supported by the NICIMS observed for derivatised [$^{13}C_2$]glycine, where it was found that a loss of 29 a.m.u. corresponded to $[M-{}^{13}CO]^-$. In addition, other losses commonly observed were 148 a.m.u. (due to loss of PFP + H) and 20 a.m.u. (due to loss of HF). The principle ions in the mass spectra of the TFE–PFP derivatives of the amino acids are also shown in Table I; these derivatives had similar fragmentation patterns to the HFIP–PFP derivatives but, in this case, ions produced by loss of CO were of low abundance.

For each group of compounds studied quantitation was carried out by determining the ratio of the selected ion of the derivative of the endogenous compound in the derivatised biological extract to that of the selected ion of the deuteriated internal standard. Day-to-day instrumental variation was corrected for by measuring the corresponding ratio obtained from a derivatised 1:1 mixture of the deuteriated standards and non-deuteriated compounds. Calibration curves for all substances analysed were linear over the range examined (correlation coefficients $>0.99$). Blank samples were processed prior to each analysis to ensure that reagents were not contaminated by standard solutions and locust nervous tissue was also processed without the addition of deuteriated internal standards to rule out any possibility that these might contribute significantly to the endogenous substances present. The concentration of amino acids, phenolamines, catecholamines, 5-HT and N-acetyl metabolites are listed in Table II.

TABLE II

AMOUNTS OF AMINO ACIDS, PHENOLAMINES, CATECHOLAMINES AND N-ACETYL METABOLITES IN THE VENTRAL THORACIC NERVOUS SYSTEM OF THE LOCUST, *SCHISTOCERCA GREGARIA*

| Compound | Concentation (ng per tissue) | *n* |
|---|---|---|
| Phenylalanine | 194 ± 81 | 10 |
| Tyrosine | 347 + 88 | 10 |
| DOPA | 11 ± 11 | 10 |
| *p*-Tyramine | 32.2 ± 13.8 | 17 |
| *p*-Octopamine | 2.6 ± 0.5 | 17 |
| Dopamine | 6.6 ± 2.5 | 17 |
| Noradrenaline | 0.2 ± 2.5 | 17 |
| 5-HT | 1.4 ± 0.9 | 9 |
| N-Acetyltyramine | 2.0 ± 0.7 | 15 |
| N-Acetyloctopamine | 1.0 ± 0.3 | 15 |
| N-Acetyldopamine | 6.8 ± 8.5 | 10 |
| N-Acetyl-5-HT | 0.07 ± 0.02 | 6 |
| Acidic metabolites | Not detected | |

The quantities of amino acids in nerve cords were determined using selected-ion monitoring (SIM) and monitoring the most abundant ions in the mass spectra of the HFIP–PFP derivatives. The concentrations of phenylalanine, tyrosine and DOPA in locust nerve cords are shown in Table II. In view of their selectivity and sensitivity for the analysis of phenylalanine, tyrosine and DOPA the HFIP–PFP derivatives were chosen for the identification and quantitation of amino acids in biological samples. TFE–PFP derivatives were used to confirm the identity of the amino acids since the GC retention times and mass spectra were changed in a predictable manner. A direct comparison of our results for amino acids and those previously published is not possible due to the differences in the quantitative units. However, the relative values compare favourably with those in previous reports in insects in that the concentration of tyrosine is approximately double that of phenylalanine. To the best of our knowledge, there are no previous reports concerning the concentration of DOPA in the locust nervous system.

GC and MS properties were determined for a variety of biogenic amines as their DTFMB–TMS and DTFMB–TBDMS derivatives [33]. The concentrations of biogenic amines in locust ventral thoracic nerve cords are shown in Table II. Typical traces of derivatised extracts of biogenic amines (as both DTFMB–TMS and DTFMB–TBDMS derivatives) from locust ventral nerve cords are shown in Figs. 1 and 2. The most significant finding regarding the concentrations of phenolamines (see Table II) in locust nervous tissue was the relatively high concentrations of *p*-tyramine. Shafi *et al.* [11], in our laboratories, used GC–NICIMS to determine 3.4 ng of *p*-tyramine in the brain of the cockroach (*Periplaneta americana*): a figure which was greater than ten times that reported previously by Martin *et al.* [35] using HPLC–ED. Studies on the biosynthesis of biogenic amines in insects reveal the importance of tyramine as a biosynthetic intermediate. For example, the work *in vitro* with the isolated ganglia of the moth, *Manduca sexta*, showed that the label from [$^{14}$C]tyrosine accumulated in tyramine to a greater extent than in either dopamine or octopamine [36]. In view of the apparent absence of a specific physiological role for tyramine in insect nervous tissue, further work will be required to determine whether or not the high concentration of *p*-tyramine observed here represents a function of greater significance than that of a biosynthetic intermediate.

In contrast to recent work by Thonoor *et al.* [37] which demonstrated that all the positional isomers of octopamine (together with *m*- and *p*-synephrine) occurred naturally in mammals, *o*- and *m*- octopamine and the isomeric synephrines were absent from the tissues analysed.

The mass spectral data obtained under NICI conditions for the TFA and PFP derivatives of the N-acetates of biogenic amines are shown in Table III. The mass spectra consisted of a few large ions produced by the loss of PFP or TFA groups. The PFP and TFA derivatives of N-acetyl-*p*-octopamine afforded mass spectra in which the ion current was largely carried by reagent-specific ions due to dissociative resonance capture; we have found that this usually occurs when there is

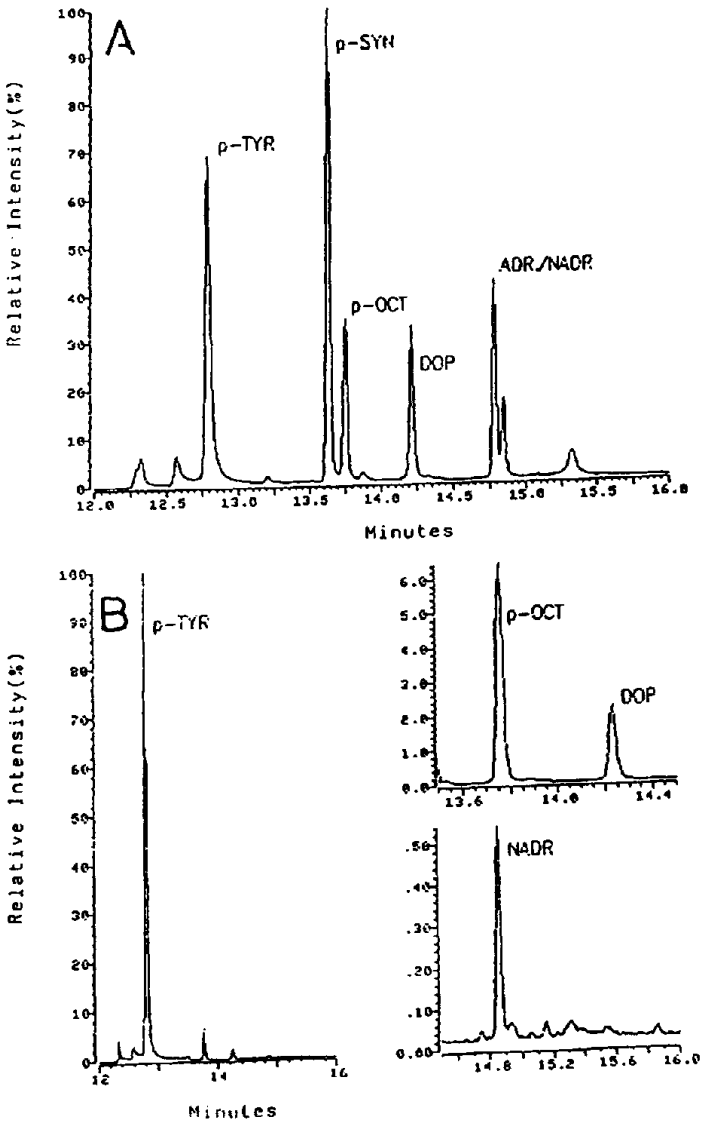


Fig. 1. (A) SIM trace of a standard mixture (20 ng each) of *p*-tyramine (p-TYR, *m/z* 449), *p*-synephrine (p-SYN, *m/z* 551), *p*-octopamine (p-OCT, *m/z* 537), dopamine (DOP, *m/z* 537), adrenaline (ADR, *m/z* 639) and noradrenaline (NADR, *m/z* 625) as DTFMB–TMS derivatives. (B) SIM trace of *p*-tyramine (p-TYR, *m/z* 449), *p*-octopamine (p-OCT, *m/z* 537), dopamine (DOP, *m/z* 537) and noradrenaline (NADR, *m/z* 625) extracted from a single locust ventral thoracic nerve cord as DTFMB–TMS derivatives.

an aliphatic (particularly benzylic) hydroxyl group present in the molecule. The proportion of the ion current carried by the molecular ion was increased when the source temperature in the instrument was decreased to 100°C, which was used as the source temperature in analyses of the acetates. The use of an isotopomeric

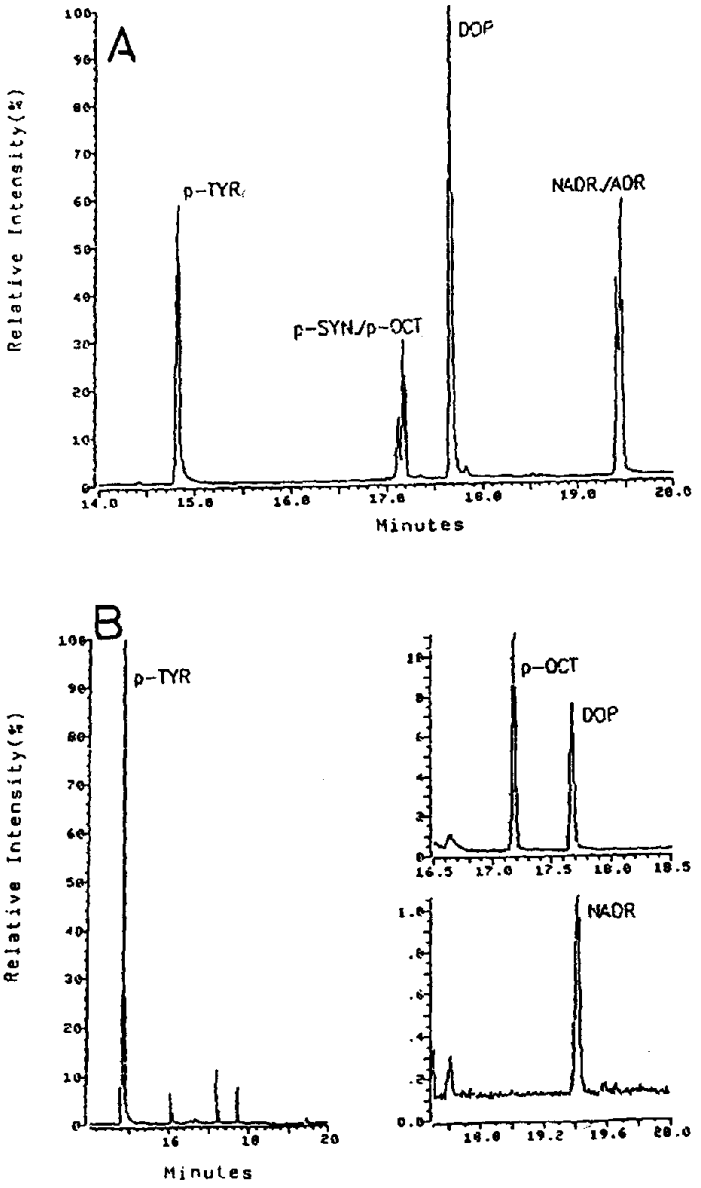


Fig. 2. (A) SIM trace of a standard mixture (20 ng each) of *p*-tyramine (*m/z* 491), *p*-synephrine (*m/z* 635), *p*-octopamine (*m/z* 621), dopamine (*m/z* 621), noradrenaline (*m/z* 751) and adrenaline (*m/z* 765) as DTFMB–TBDMS derivatives. (B) SIM trace of *p*-tyramine (*m/z* 491), *p*-octopamine (*m/z* 621), dopamine (*m/z* 621) and noradrenaline (*m/z* 751) extracted from a single locust thoracic nerve cord as DTFMB–TBDMS derivatives. For abbreviations see Fig. 1.

TABLE III

PRINCIPAL IONS IN THE MASS SPECTRA OF THE PFP AND TFA DERIVATIVES OF N-ACETYL METABOLITES OF BIOGENIC AMINES

| Compound | *I* value | Base peak | Other significant ions |
|---|---|---|---|
| *PFP* | | | |
| Tyramine N-acetate | 1547 | 471 | 451 (30) |
| Dopamine N-acetate | 1616 | 186 | 486 (45) |
| *p*-Octopamine N-acetate | 1559 | 163 | 633 (25) |
| *TFA* | | | |
| Tyramine N-acetate | 1524 | 371 | |
| Dopamine N-acetate | 1588 | 386 | 483 (29) |
| *p*-Octopamine N-acetate | 1541 | 113 | 483 ( 7) |

internal standard is essential in SIM when dissociative resonance capture predominates. This is to compensate for fluctuations in the proportion of ion current carried by the ion being monitored; we have found that an analogue internal standard produces very innaccurate results in these circumstances.

A problem observed with the use of PFP derivatives was that there was substantial, inconsistent exchange of deuterium when the deuteriated internal standards were derivatised on a small scale ($<20$ ng). Reduction in the time/temperature conditions for derivative formation decreased the amount of exchange but not to a level suitable for accurate quantitation; consequently TFA derivatives were used for quantitative work since TFAA did not promote deuterium exchange. Derivatisation of extracts with PFP in the absence of deuteriated internal standards was used to confirm the identity of the N-acetates.

The amounts of *N*-acetylated metabolites in locust ventral thoracic nerve cords are listed in Table II. It has been reported that the amounts of NAD in the cerebral ganglion of the cockroach, *Periplaneta americana*, were 2.10 μg/g wet weight [38] and 2.38 μg per organ [39] using HPLC–ED. In the former publication [38] the authors stated that N-acetyldopamine acted as its own internal standard when in fact is was used as an external standard; this is not a satisfactory method for quantitation. Recently quantitative results for N-acetyloctopamine were reported [22] for cockroach haemolymph, following the injection of *p*-octopamine into the animals; however, the method used for quantitation was not reported. To the best of our knowledge there are no reliable reports on the concentration of N-acetyltyramine in invertebrate nervous systems.

GC and MS properties were determined for the acidic metabolites of biogenic amines as their Ac-DTFMBBr [30] derivatives. The derivatives of acidic metabolites of biogenic amines produced mass spectra where the ion current was largely carried by a single ion resulting from the loss of the DTFMB moiety from the derivative. However, acidic metabolites of biogenic amines could not be detected

as their Ac-DTFMBBr derivatives in locust ventral nerve cords (single tissues or pooled samples of five nerve cords) above the limit of detection of the method (*ca.* 0.5 ng per tissue). This, together with previous results (*vide supra*), confirms that N-acetylation is the principal route of monoamine inactivation in insect nervous systems.

GC and MS properties were determined for (−)-*m*-, (−)-*p*- and (+)-*p*-octopamine as their (−)-HFBPAL–TBDMS derivatives [40]. The derivatives gave mass spectra where the base peak (*m/z* 704) was due to loss of 20 a.m.u. ($[M-HF]^-$) from the molecular ion (Fig. 3). The (−)-HFBPAL–TBDMS derivative proved to be suitable for the small scale (< 10 ng) analysis of *p*-octopamine. The limits of detection for the derivative were established at *ca.* 1 ng. (−)-*m*-Octopamine was used as an internal standard in analysis of *p*-octopamine in nerve cords, which were extracted and the amines converted to the (−)-HFBPAL–TBDMS derivatives followed by monitoring the ion of *m/z* 704 (see Fig. 4). There is little evidence of the presence of (+)-*p*-octopamine in the extract with a definitive peak for the (−)-isomer; the presence of *ca.* 5% of the (−)-HFBPAL–TBDMS derivative of the (+)-isomer of *m*-octopamine is probably largely due to incomplete resolution of the material but it is not possible to rule out other sources such as the (−)-phenylalanine used to prepare the (−)-HFBPALCl or the derivatisation procedures. Thus it is not possible to say whether or not the method *per se* is suitable for the accurate determination of enantiomeric purity but it is very effective for determining the relative configurations of biogenic amines *in vivo*. Thus it was established unequivocally that the endogenous *p*-octopamine in locust ventral thoracic nerve cords is the (−)-isomer. This possesses the (*R*)-configuration on the basis of X-ray crystallographic and circular dichroism studies previously conducted on enantiomers of *p*-synephrine, *p*-octopamine and a number of related compounds [31].

Thus we have developed highly sensitive and specific methodologies for the identification of endogenous substances in biological systems where variation in

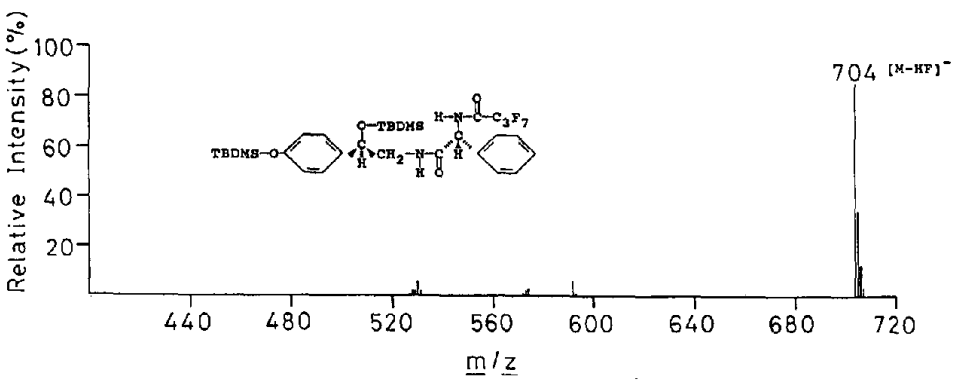


Fig. 3. NICI mass spectrum of the (−)-HFBPAL–TBDMS derivative of (*R*)-(−)-*p*-octopamine.

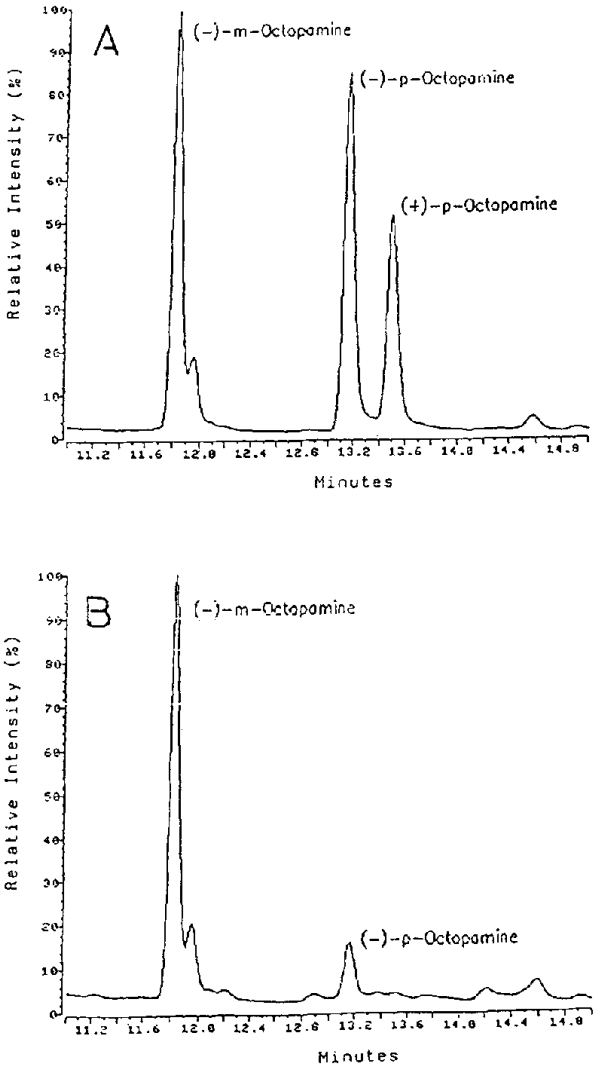


Fig. 4. SIM trace (monitoring *m/z* 704) for the (−)-HFBPAL–TBDMS derivative of (+)- and (−)-*p*-octopamine (20 ng each). (−)-*m*-Octopamine (10 ng) was added as an internal standard. (B) SIM trace (monitoring *m/z* 704) for the (−)-HFBPAL–TBDMS derivative of *p*-octopamine extracted from locust ventral nerve cord. (−)-*m*-Octopamine (10 ng) was added as an internal standard.

derivatisation reagent allowed a shift in the *m/z* values of characteristic ions and retention times of a given compound. These permit their unequivocal identification in contrast to methods such as HPLC, which rely on retention time alone.

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