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Identification and quantitation of N-acetyl metabolites of biogenic amines in the thoracic nervous system of the locust, *Schistocerca gregaria*, by gas chromatography–negative-ion chemical ionisation mass spectrometry

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ABSTRACT

The N-acetylated metabolites of *p*-tyramine, *p*-octopamine and dopamine were identified unambiguously and quantitatively determined in a single ventral thoracic nerve cord of the locust, *Schistocerca gregaria*, by gas chromatography–negative-ion chemical ionisation mass spectrometry (GC–NICIMS). Deuterium-labelled analogues of each compound were added to a single ventral thoracic nerve cord in acetonitrile· the tissue was homogenised and the suspension centrifuged The solvent was removed from the supernatant and the resultant residue was derivatised with trifluoroacetic anhydride. Under negative-ion chemical ionisation conditions, the trifluoroacetyl derivatives produced ions which were sufficiently abundant to be suitable for selected-ion monitoring This method is highly specific and gave a limit of detection below the picogram levels N-Acetyl-5-hydroxytryptamine was determined using a previously published GC–NICIMS technique [S. P Markey, R. W. Colburn and J N Johannessen, *Biomed. Mass Spectrom* , 7 (1981) 301]. The concentrations of N-acetyltyramine, N-acetyloctopamine, N-acetyldopamine and N-acetyl-5-hydroxytryptamine in locust thoracic nerve cords were, respectively, 1.86 ± 0.71, 1 13 ± 0 34, 6 77 ± 8.48 and 0.07 ± 0.02 ng per tissue.

INTRODUCTION

It has been proposed that *p*-octopamine, dopamine and 5-hydroxytryptamine (5-HT) are neurotransmitters in the central nervous system of insects [1–4]. In our earlier work we established unequivocally by gas chromatography–negative-ion chemical ionisation mass spectrometry (GC–NICIMS) that the principal amines in cockroach brain [5] and locust thoracic nerve cords [6] 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 mecha-

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nisms have been studied in relation to the inactivation of monoamines in insects which include N-acetylation [7–10], oxidative deamination [11,12], O-sulphate or β-alanyl conjugation [13,14] and sodium-sensitive and sodium-insensitive uptake mechanisms [15]. The majority of evidence accumulated favours *N*-acetylation as the principal mechanism of inactivation in these species [16].

Various sensitive but albeit non-specific methods are available for the detection of N-acetyl metabolites: from thin-layer chromatography (TLC) [7] to an enzymatic radiochemical assay [17] to the more elegant method of high-performance liquid chromatography with electrochemical detection (HPLC–ED) [18]. The GC analysis of N-acetyldopamine (NAD), as its trimethylsilyl derivative [19], led us to develop our highly sensitive and specific GC–NICIMS technique for the identification and quantitation of N-acetyltyramine (NAT), N-acetyloctopamine (NAO) and N-acetyldopamine (NAD) in biological matrices. N-Acetyl-5-hydroxytryptamine (NA-HT) was identified and quantified using a previously published GC–NICIMS technique [20].

GC–MS has the advantage over other methodologies in that compounds may be identified unequivocally by high-resolution capillary GC combined with the monitoring of characteristic ions of high abundance in the mass spectrum of a compound. These may afford characteristic ratios of ion intensities or their m/z values may be changed in a predictable manner, to provide additional proof of identity, by the preparation of a different derivative of the same chemical class. This technique is specific and allows for the first time the unambiguous measurement of NAT, NAO and NAD in the tissues of individual insects. Quantitative methods for the determination of NAD may be of clinical interest in view of the reports of NAD (using paper chromatography, TLC and high-voltage paper electrophoresis) in patients with neuroblastoma [21].

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 100°C, 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) capillary column, helium carrier gas was used with a head pressure of 0.54 bar.

The GC conditions were as follows: injector temperature 250°C, transfer line temperature 220°C, the oven temperature was maintained at 80°C for 1 min then programmed at 5°C/min to 300°C. Injections were made using a Grob splitless injection system.

Materials and reagents

All solvents used in extraction were HPLC grade (Rathburn, Walkerburn, U.K.). Chemicals were obtained from the following sources: *p*-tyramine hydrochloride, dopamine hydrochloride, (±)-*p*-octopamine hydrochloride, deuterium oxide, sodium acetate, acetic anhydride, hexadeuteriated sodium acetate, hexadeuteriated acetic anhydride, N-acetyl-5-hydroxytryptamine, trifluoroacetic anhydride (TFAA), pentafluoropropionic anhydride (PFPA), potassium tetraborate tetrahydrate from Aldrich (Gillingham, U.K.); NAD from Sigma (Poole, U.K.); 4,6-difluoro-5-hydroxytryptamine was kindly donated by Dr. K. L. Kirk, Department of Health and Human Services, NIH (Bethesda, MD, U.S.A.).

Synthesis of standards and deuterium-labelled internal standards

N-Acetyl-p-octopamine. *p*-Octopamine hydrochloride (1.68 g) was dissolved in water (10 ml), acetic anhydride (1.07 g) was added followed at once by sodium acetate trihydrate (1.30 g) [22]. The colourless viscous reaction mixture was shaken vigorously for 1.5 h using a mechanical shaker, and excess solvent was removed under vacuum. The resultant yellow viscous liquid failed to crystallise from ethyl acetate or an acetone–ether mixture. TLC (Merck Kieselgel $60F_{254}$; ethyl acetate) revealed that the product was a mixture of *p*-octopamine and its N-acetylated derivative. These were separated on a column (12 cm × 5 cm) of silica gel (7–200 mesh, 45 g) which was eluted with ethyl acetate–acetone (3:1); the fractions were monitored by TLC (Merck Kieselgel $60F_{254}$; ethyl acetate). The fractions containing N-acetyl-*p*-octopamine were combined and the solvent was removed under vacuum to yield a colourless crystalline residue. This was recrystallised from ethyl acetate to yield needles (0.45 g, 25.8%, m.p. 124–125°C, lit. [21] 145°C). Found: C, 61.66%; H, 6.89%; N, 7.36%; calculated for $C_{10}H_{13}NO_3$: C, 61.54%; H, 6.67%; N, 7.18%. The infrared spectrum (Nujol) showed a single carbonyl stretching vibration (s) at 1650 cm^{-1}. The ^{1}H NMR spectrum [250 MHz; $(CD_3)_2SO$] indicated the following signals: 1.8 (3H, s, CH_3), 3.1 (1H, ddd, $CHOHCH_2$, $J_{AB}=13.2$, 8.0, 5.1 Hz), 3.2 (1H, ddd, $CHOHCH_2$, $J_{AB}=13.2$, 6.2, 5.1 Hz), 4.5 (1H, ddd, $CHOH$, $J=4.0$, 8.0, 6.2 Hz), 5.2 (1H, d, $CHOH$, $J=4.0$ Hz), 6.7 (2H, AA′ BB′, Ar-H, $J=8$ 6 Hz), 7.1 (2H, AA′ BB′, Ar-H, $J=8.6$ Hz), 7.9 (1H, t, NH, $J=5.1$ Hz), 9.3 (1H, s, Ar-OH) ppm. When the water peak at 3.3 ppm was irradiated, signals at 5.2 and 9.3 ppm collapsed due to saturation transfer.

N-Acetyl-p-tyramine. The synthesis of N-acetyl-*p*-tyramine was carried out in a manner essentially the same as that described above with the exception of the column chromatography procedure. After the removal of excess solvent, the residue was washed several times with toluene and ethyl acetate to remove excess acetic anhydride. The crystals which formed, after removal of excess solvent, were recrystallised from acetone to yield blunt needles (0.38 g, 21%, m.p. 133–134°C, lit. [23] 132.5–133.5°C). Found: C, 66.95%; H, 7.39%; N, 7.64%; calculated for $C_{10}H_{13}O_2$: C, 67.04%; H, 7.31%; N, 7.82%. The infrared spectrum (Nujol)

showed a single carbonyl stretching vibration at 1640 cm^{-1} and the ^1H NMR spectrum [250 MHz, $(CD_3)_2SO$] indicated signals at: 1.8 (1H, s, CH_3), 2.6 (2H, t, Ar-CH_2-CH_2, J=7.1 Hz), 3.2 (2H, dt, CH_2-CH_2-NH, J=6.4, 7.1 Hz), 6.8 (2H, AA′ BB′, Ar-*H*, J=8.6 Hz), 7.0 (2H, AA′ BB′, Ar-*H*, J=8.6 Hz), 7.9 (1H, t, N*H*, J=6.4 Hz), 9.2 (1H, s, O*H*) ppm. When the water peak at 3.4 ppm was irradiated the peak at 9.2 ppm collapsed due to saturation transfer.

Trideuterio-N-acetyloctopamine. Trideuterio-N-acetyloctopamine was synthesised in a manner similar to that described above for N-acetyl-*p*-tyramine with the exception that deuterium oxide, hexadeuteriated acetic anhydride and hexadeuteriated sodium acetate were used instead of the corresponding undeuteriated reagents. The resultant colourless crystals were recrystallised from ethyl acetate to yield a microcrystalline solid (0.28 g, 32%, m.p. 123–124°C). The ^1H NMR spectrum [90 MHz; $(CD_3)_2SO$] showed that the signals corresponding to the protons of the acetyl group were absent, and the GC–NICIMS of the trifluoroacetyl (TFA) derivative using $M^{\bar{\cdot}}$ (*m/z* 483–486) indicated $M^{\bar{\cdot}}d_3$ (100%) with none of the other ions being present in the mass spectrum.

Trideuterio-N-acetyltyramine. This was synthesised as described above to yield colourless needles (0.1 g, 11%, m.p. 129–131°C). The ^1H NMR spectrum [90 MHz; $(CD_3)_2SO$] showed that the signals corresponding to the protons of the acetyl group were absent, and the GC–NICIMS of the TFA derivative using $M^{\bar{\cdot}}$ (*m/z* 371–374) indicated $M^{\bar{\cdot}}d_3$ (100%) with none of the other ions being present in the mass spectrum.

Trideuterio-N-acetyldopamine. Dopamine hydrochloride (0.5 g) was dissolved in potassium tetraborate tetrahydrate (1 *M*, 10 ml) [24] and reacted with hexadeuteriated acetic anhydride (400 μl). Stirring was initiated and continued for a further hour. The reaction mixture was then acidified using hydrochloric acid (6 *M*) and extracted with ethyl acetate (2 × 25 ml). Excess solvent was removed under vacuum, and the residue was washed several times with toluene and ethyl acetate. The resultant colourless powder which remained, after the removal of excess solvent, was crystallised from ethyl acetate. However, the trideuterio-N-acetyldopamine proved to be very hygroscopic and formed a gum on filtration. The compound was dried over phosphorous pentoxide in a vacuum but subsequently crystallisation could not be induced from ethyl acetate, ethyl acetate–hexane or chloroform. Trideuterio-N-acetyldopamine remained in solution in ethyl acetate and an aliquot (200 μl, *ca.* 1 μg/μl) was removed; excess solvent was removed under a stream of nitrogen. The residue was derivatised using TFA, and the GC–NICIMS of the TFA derivative using $M^{\bar{\cdot}}$ (*m/z* 386–389) indicated $M^{\bar{\cdot}}d_3$ (100%) with none of the other ions being present in the mass spectrum.

Extraction and derivatisation

Ventral thoracic nerve cords were dissected from male and female two- to seven-day-old adult locusts, *Schistocerca gregaria*, obtained from crowded laboratory cultures fed on wheat seedlings and were immediately frozen on dry ice.

They were transported overnight in dry ice from the University of Cambridge and stored at $-20°C$ prior to analysis. Prolonged storage did not appear to have any adverse affects on the concentrations of N-acetylated metabolites in the locust nerve cords. A standard solution [20 μl, equivalent to 20 ng each of trideuterio-N-acetyltyramine (NAT-d_3), trideuterio-N-acetyloctopamine (NAO-d_3) and trideuterio-N-acetyldopamine (NAD-d_3)] was added to acetonitrile (0.5 ml) containing a single ventral thoracic nerve cord in a ground-glass homogeniser. The tissue was homogenised and the homogenate centrifuged for 30 min at 2500 *g* (4500 rpm). The supernatant was transferred to a screw-capped vial (1 ml), and the solvent

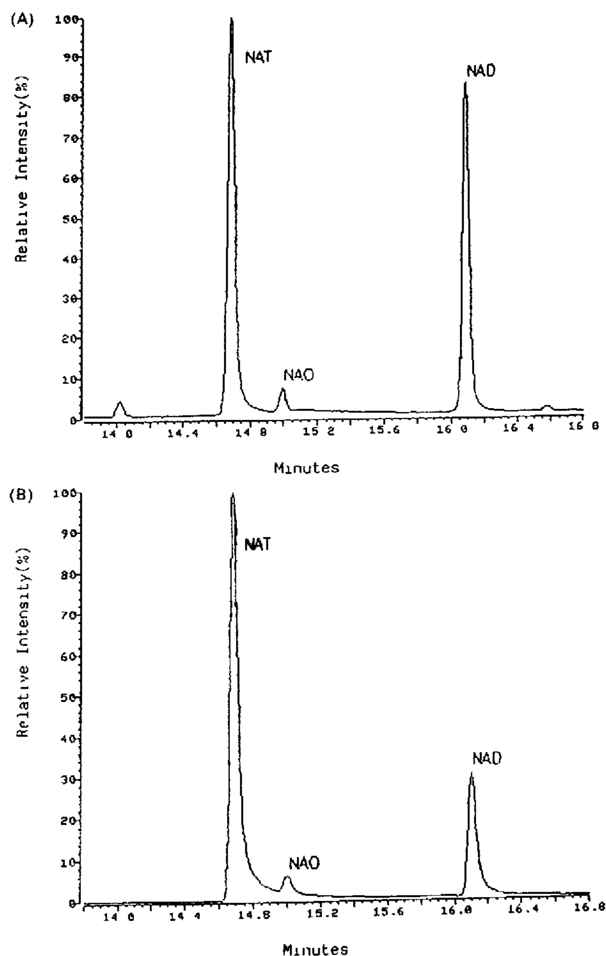

Fig 1 (A) Selected-ion monitoring trace of a standard mixture (20 ng each) of N-acetyl-*p*-tyramine (*m*/*z* 371), N-acetyl-*p*-octopamine (*m*/*z* 483) and N-acetyldopamine (*m*/*z* 386) as TFA derivatives. (B) Selected-ion monitoring trace of TFA derivatives of N-acetyl-*p*-tyramine (*m*/*z* 371), N-acetyl-*p*-octopamine (*m*/*z* 483) and N-acetyldopamine (*m*/*z* 386) from an extract of a locust thoracic nerve cord.

was removed under a stream of nitrogen. Dried tissue extracts or standards were heated with TFAA (50 μl) for 10 min at 100°C in a screw-capped vial. The excess TFAA was evaporated under a stream of nitrogen and the residue dissolved in ethyl acetate (20 μl). For the formation of PFP derivatives, the extracts or standards were heated with PFPA (50 μl) for 10 min at 100°C, and the excess solvent was removed under a stream of nitrogen. The residue was dissolved in ethyl acetate (20 μl) prior to analysis by GC–NICIMS.

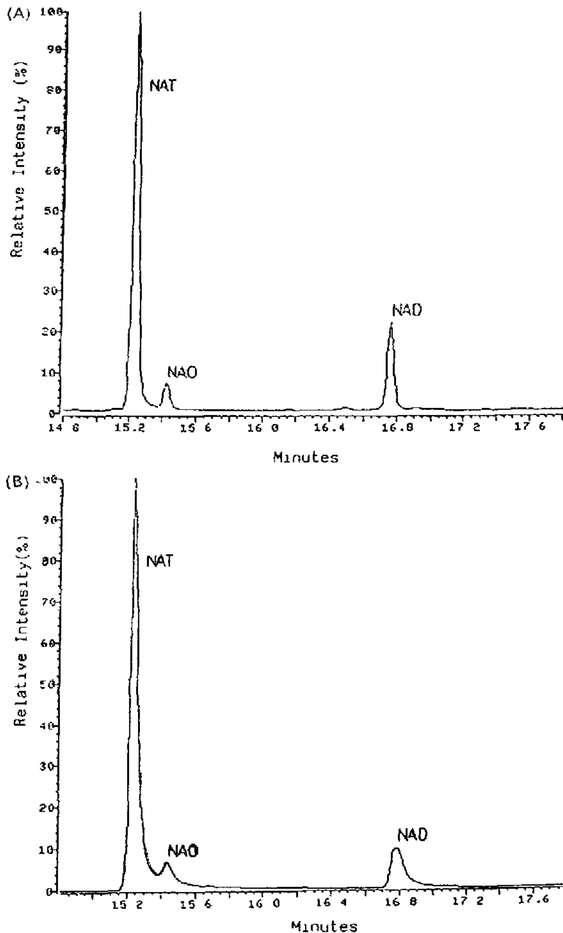

Fig. 2. (A) Selected-ion monitoring trace of a standard mixture (20 ng each) of N-acetyl-*p*-tyramine (*m/z* 471), N-acetyl-*p*-octopamine (*m/z* 633) and N-acetyldopamine (*m/z* 486) as PFP derivatives. (B) Selected-ion monitoring trace of PFP derivatives of N-acetyl-*p*-tyramine (*m/z* 471), N-acetyl-*p*-octopamine (*m/z* 633) and N-acetyldopamine (*m/z* 486) from an extract of a locust thoracic nerve cord

RESULTS AND DISCUSSION

Fig. 1 shows typical GC–MS responses to the TFA derivatives of NAT, NAO and NAD in a 1:1 standard mixture (A) and those from a preparation of a locust thoracic nerve cord (B). Identification of NAT, NAO and NAD in locust nervous tissue was carried out by establishing that the retention times (NAT, 14.68 min; NAO, 14.99 min; NAD, 16.09 min) and the selected ions (NAT, $M^{\bar{\cdot}}$, *m/z* 371; NAO, $M^{\bar{\cdot}}$, *m/z* 483; NAD, $[M-CF_3CO]^{\bar{\cdot}}$, *m/z* 386) of the TFA derivatives of endogenous NAT, NAO and NAD were identical to those of the derivatised authentic standard substances. The same identification procedure was carried out on the corresponding PFP derivatives (Fig. 2A and B). This method of double derivatisation was necessary because the fragmentation pattern of these derivatives in the NICI mode rendered use of the ratio of intensities of ions in the same spectrum less reliable than that derived by electron impact-induced ionisation.

Quantitation was carried out by determining the ratio of the intensity of the selected ion (NAT, *m/z* 371; NAO, *m/z* 483; NAD, *m/z* 386) of the TFA derivative of the endogenous compound in the derivatised biological extract to that of the selected ion of the added deuteriated internal standard (NAT-d_3, *m/z* 374; NAO-d_3, *m/z* 486; NAD-d_3, *m/z* 389). 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 were linear over the range examined (correlation coefficients >0.98). 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 N-acetyl metabolites present.

PFP derivatives of biogenic amines are widely used in GC analyses because they are easily formed and are stable for several hours [25,26]. However, the sole use of PFP derivatives for the analyses of the N-acetyl metabolites of these amines proved to be unsuitable. During initial method development the source temperature was set at 140°C but this resulted in the $M^{\bar{\cdot}}$ of NAO-PFP carrying only 6% of the negative-ion current The base peak was due to the formation of the reagent-specific ion ($C_2F_5CO_2^-$, *m/z* 163), formed because NAO-PFP underwent dissociative resonance electron capture followed by cleavage of the benzylic C–O bond. On reducing the source temperature to 100°C we observed that the $M^{\bar{\cdot}}$ of NAO-PFP now carried 25% of the negative-ion current resulting in a greatly improved limit of detection. The significant ions of the PFP derivatives of NAT, NAO and NAD are shown in Table I.

Another problem observed with the use of PFP derivatives was that there was substantial, inconsistent exchange of deuterium when deuteriated internal standards were derivatised on a small scale (20 ng or less). Reduction in the time/temperature conditions for derivative formation decreased the amount of ex-

TABLE I

KOVATS' INDICES AND INTENSITIES OF RELEVANT IONS OF PFP AND TFA DERIVATIVES OF N-ACETYLTYRAMINE, N-ACETYLOCTOPAMINE AND N-ACETYLDOPAMINE

Derivative	Kovats' index	Molecular mass	Other significant ion (m/z)
N-Acetyltyramine-PFP	1547	471 (100%)	451 (30%)
N-Acetyloctopamine-PFP	1559	633 (25%)	163 (100%)
N-Acetyldopamine-PFP	1616	633 (0%)	186 (100%)
			486 (45%)
N-Acetyltyramine-TFA	1524	371 (100%)	–
N-Acetyloctopamine-TFA	1541	483 (5%)	113 (100%)
N-Acetyldopamine-TFA	1588	483 (29%)	386 (100%)

change but not to a level suitable for accurate quantitation (Table II). This inconsistent exchange of deuterium by hydrogen may explain why the calibration curves for PFP derivatives were non-linear. However, the PFP derivatives still proved useful for confirmation of the presence of NAT, NAO and NAD in biological tissues. This was carried out by establishing that the retention times (NAT, 15.23 min; NAO, 15.42 min; NAD, 16.76 min) and the selected ions (NAT, $M^{\bar{\cdot}}$, m/z 471; NAO, $M^{\bar{\cdot}}$, m/z 633, NAD, $[M-C_2F_5CO]^-$, m/z 486) of the PFP derivatives of endogenous NAT, NAO and NAD were identical to those of the derivatised authentic standard substances. Fig. 3A and B illustrate the complete mass spectra of NAT-PFP and NAT-d_3-PFP, respectively. The use of TFA derivatives solved the problems encountered with PFP derivatives. The characteristic ions of TFA derivatives of NAT, NAO and NAD are listed in Table I, and Fig. 4A and B illustrate the complete mass spectra of NAT-TFA and NAT-d_3-

TABLE II

PERCENTAGE DEUTERIUM EXCHANGE BY HYDROGEN WHEN N-ACETYLATED AMINES ARE EXTRACTED AND DERIVATISED ON A SMALL (20 ng) SCALE (100°C/10 min)

Compound	Percentage undeuteriated
N-Acetyltyramine-PFP	10.8
N-Acetyloctopamine-PFP	14.8
N-Acetyldopamine-PFP	4.6
N-Acetyltyramine-TFA	2.4
N-Acetyloctopamine-TFA	1.1
N-Acetyldopamine-TFA	1.2

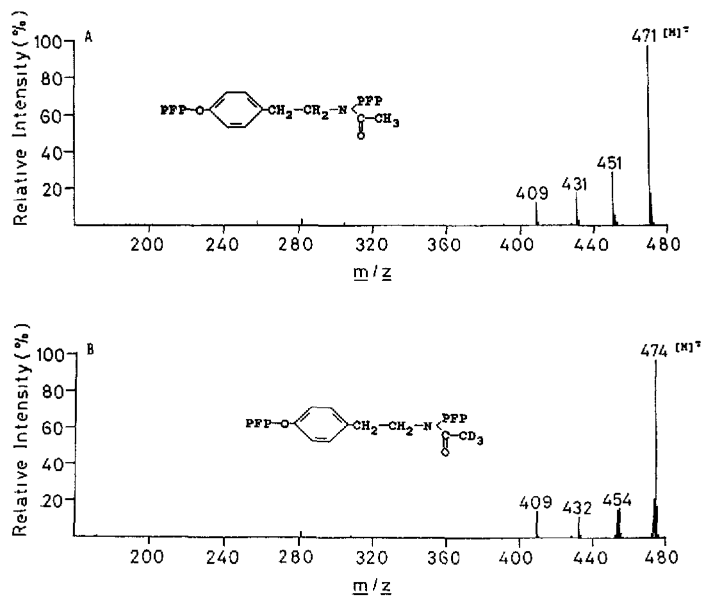

Fig 3 (A) NICI mass spectrum of N-acetyl-*p*-tyramine, PFP derivative. (B) NICI mass spectrum of trideuterio-N-acetyl-*p*-tyramine, PFP derivative

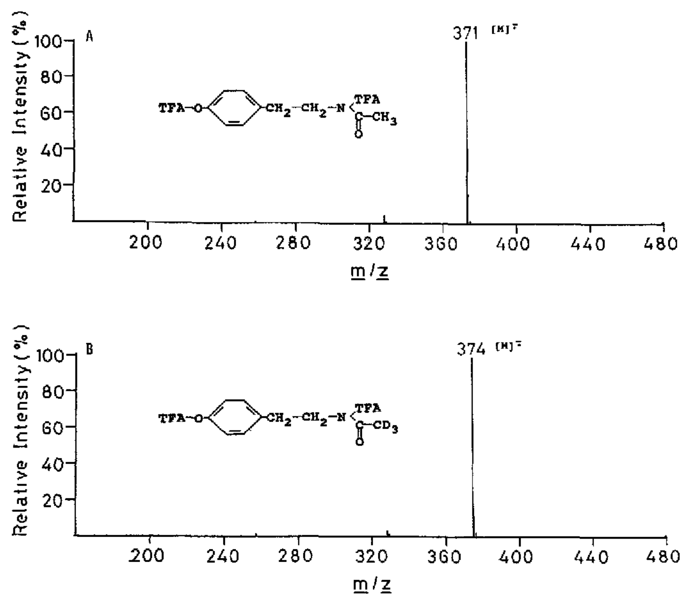

Fig 4. (A) NICI mass spectrum of N-acetyl-*p*-tyramine, TFA derivative. (B) NICI mass spectrum of trideuterio-N-acetyl-*p*-tyramine, TFA derivative

TFA, respectively. The $M^{\bar{\cdot}}$ of NAO-TFA carried 5% of the negative-ion current but this proved to be suitable for the identification and quantitation of NAO in a single ventral thoracic nerve cord. Exchange of deuterium by hydrogen was observed during the formation of TFA derivatives but the amount was negligible (Table II). Calibration curves for TFA derivatives were linear over the range examined with correlation coefficients >0.98. The TFA derivatives were used for routine identification and quantitation of NAT, NAO and NAD in biological tissues.

The amounts of NAT, NAO and NAD were determined by comparison of the ratio of intensities of $M^{\bar{\cdot}}d_0/M^{\bar{\cdot}}d_3$ measured for the derivatised biological sample with that arising from the derivatised mixture of equal quantities of authentic deuteriated and non-deuteriated substances. The results are summarised in Table III.

NA-HT was determined in locust nervous tissue using the method of Markey *et al.* [20], which involves the formation of a spirocyclic PFP derivative and, in the present work, 4,6-difluoro-5-hydroxytryptamine was used as an internal standard for quantitative purposes. Identification of NA-HT in the nervous tissue was confirmed by establishing that the retention time and ratio of the intensities of the $[M-HF]^{\bar{\cdot}}$ (*m/z* 382) and $[M-2HF]^{\bar{\cdot}}$ (*m/z* 362) ions of endogenous NA-HT were identical, within experimental error, to those of the derivatised standard compound. Fig. 5 shows the GC–MS responses for derivatised NA-HT in a standard mixture (A) and that from a locust nerve cord (B). Quantitation of NA-HT was carried out by determining the ratio of the combined ion currents carried by the selected ions ($[M-HF]^{\bar{\cdot}}$, *m/z* 382; $[M-2HF]^{\bar{\cdot}}$, *m/z* 362) in the derivatised biological extract to that of the combined current arising from the selected ions of the added internal standard, 4,6-difluoro-5-hydroxytryptamine ($M^{\bar{\cdot}}$, *m/z* 452; $[M-HF]^{\bar{\cdot}}$, *m/z* 432). The concentration of NA-HT in locust nervous tissue is listed in Table III.

NAD [27] and to a lesser extent NAT [28] are considered to be important intermediates in the sclerotisation of the insect cuticle. The role of N-acetylation in the sclerotisation process is well accepted; however, it has been widely reported

TABLE III

CONCENTRATIONS OF N-ACETYL METABOLITES IN LOCUST VENTRAL THORACIC NERVE CORD

Compound	Concentration (ng per tissue)	*n*
N-Acetyltyramine	1 86 ± 0 71	15
N-Acetyloctopamine	1.13 ± 0.34	15
N-Acetyldopamine	6 77 ± 8 48	12
N-Acetyl-5-hydroxytryptamine	0 07 ± 0.02	6

that N-acetylated biogenic amines occur in invertebrate nervous systems and their presence has been ascribed to the inactivation of neurotransmitters [7–10]. The importance of N-acetylation as a means of inactivation of biogenic amines was first noted in studies on *Drosophila* nervous tissue Tyramine was the best substrate tested for the N-acetyltransferase, which also had a significant activity towards dopamine and 5-HT [29,30]. Using TLC with radiolabelling Vaughan and Neuhoff [31] reported that N-acetylation was the major reaction of phenethylamines associated with ganglia of the nervous system of *Schistocerca*

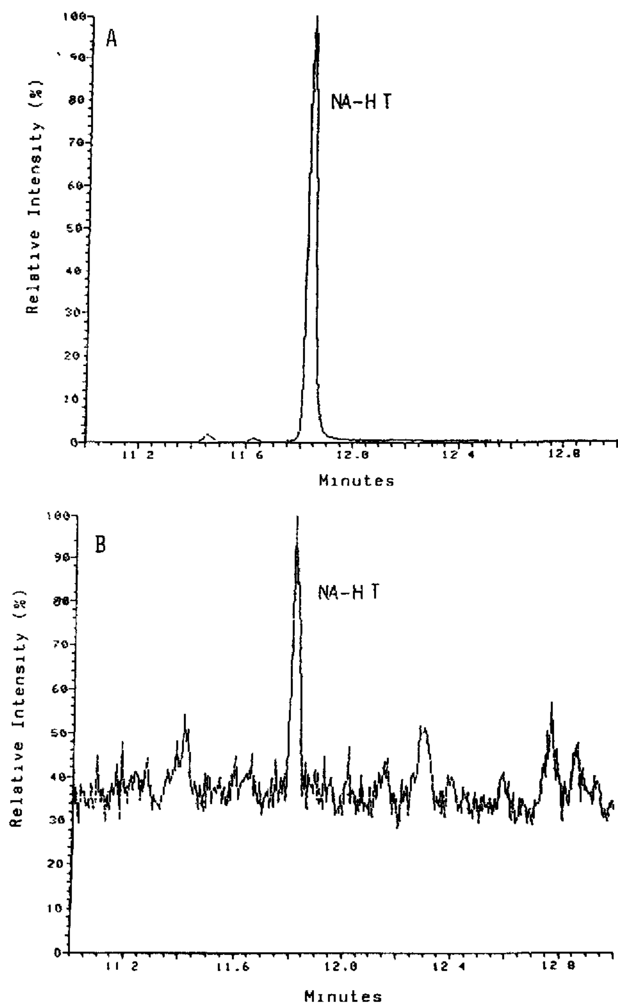

Fig. 5. (A) Selected-ion monitoring trace of a standard mixture (20 ng) of N-acetyl-5-hydroxytryptamine (*m/z* 382 and 362) as a spirocyclic PFP derivative. (B) Selected-ion monitoring trace of a spirocyclic PFP derivative of N-acetyl-5-hydroxytryptamine (*m/z* 382 and 362) from an extract of locust thoracic nerve cord.

gregaria. This view received further support when NAO, NAD and NA-HT were reported to be prominent in nerve tissue and malpighian tubules of *Locusta migratoria* using TLC [7]. The presence of NA-HT (again using TLC) has also been reported in nervous tissue from the honeybee, *Apis mellifera* [10]. More recently [32] HPLC–ED has been employed to detect the production of NA-HT in the haemolymph of adult cockroaches, after the injection of 5-HT, but the method of quantitation was not stated. 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 [33] and 2.38 μg per organ [34] using HPLC–ED. In the former publication [33] the authors stated that NAD acted as its own internal standard when in fact it was used as an external standard; this is not a satisfactory method for quantitation. Recently quantitative results for NAO were reported [16] 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 NAT in invertebrate nervous systems.

Thus we have developed a highly sensitive and specific method for the identification of endogenous N-acetyl metabolites in biological systems where variation in 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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