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Identification of tryptophan and β-carboline as paralysins in larvae of the yellow mealworm, *Tenebrio molitor*

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Abstract

Acidic methanolic, whole body extracts of larval *Tenebrio molitor* (Insecta, Coleoptera) and other juvenile insects are highly toxic to adults of the same species and other species: injection causes instant paralysis to death. Referring to their dramatic effect in mature insects, the responsible compounds have been designated as "paralysins." Two paralysins have already been identified in the flesh fly, *Neobellieria bullata*, i.e., β-alanine-tyrosine (BAY) and 3-hydroxykynurenine (3HK). We report here the isolation, from larval *T. molitor*, of two additional paralysins, respectively, the essential amino acid, tryptophan (Trp), and the saturated β-carboline, 1,2,3,4,-tetrahydro-β-carboline-3-carboxylic acid (THCA).
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Chiou et al. [1] have shown that acidic methanolic extracts of insect larvae contain toxic substances, which upon injection cause a lethal effect to adults of the same species or other species. These endogenous toxic substances, apparently being widely spread over the class of insects, have been designated as paralysins, because of their immediate and observable effect upon injection. In all insects investigated, the strongest paralytic activity has been found in late instar larvae or in the early pupal stage, suggesting that the paralysins might be involved in metamorphosis. Subsequently, Chiou et al. [2] have chromatographically purified two paralysins, both soluble in organic solvents and heat stable, to homogeneity from late, last larval instar *Neobellieria bullata* (Diptera). Paralysin I and II have been identified, via mass spectrometry (MS) and nuclear magnetic resonance (NMR), respectively, as β-Ala-Tyr, a 252 Da dipeptide with an unusual N-terminal amino acid, and 3HK, a 225 Da tryptophan metabolite. Based upon their specific temporal occurrence and toxicity Chiou et al. [1,2] proposed that these paralysins are involved in metamorphosis. The toxins may influence the viability of the nervous system and neuronal remodelling or at least inactivity during metamorphosis. Accordingly, we have continued our search for paralysins in another insect species. We report here the purification and identification of two compounds present in the yellow mealworm, *Tenebrio molitor*, which are highly toxic to both adult *N. bullata* and *T. molitor*: Trp and THCA.

Materials and methods

Animals. The grey flesh fly, *N. bullata* (Diptera), was reared as described by Huybrechts and De Loof [3]. The yellow mealworm, *T. molitor* (Coleoptera), purchased as larvae from a local pet shop, was kept on bran, at 32 °C, 40% relative humidity under a photoperiod of 16/8 h light and dark. Added to the 4–5 cm layer of bran was a small amount of milk powder and brewer's yeast. Daily, the pupae and

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adults were removed from the culture and regrouped. Approximately 800 larvae were collected for bulk purification.

Crude extraction and isolation of paralytic toxins. The collected larvae were extracted in a methanol/water/acetic acid solution (90/9/1; v/v/v). After evaporation, the aqueous residue was delipidated with ethyl acetate, followed by *n*-hexane. Subsequently, solid-phase extraction was performed on Megabond Elute C_{18} cartridges (Varian, Harbour City, CA, USA) that were eluted with 25 ml each of 30%, 60%, and 90% acetonitrile (CH_3CN) in 0.1% aqueous trifluoroacetic acid (TFA). Following are the columns and operating conditions for high performance liquid chromatography (HPLC) performed on a Beckman HPLC system with a diode array detector (chromatogram constructed at 214 and 280 nm; detector range: 2 absorption units full scale (Aufs)). Solvent A, 0.1% aqueous TFA; solvent B, 50% CH_3CN in 0.1% aqueous TFA were used for all isolation steps. For the purification of the first *Tenebrio* paralysin: (1) Preparative Delta-Pak C_{18} (25×100 mm, 100 Å, 15 μm, Waters Associates, Milford, MA). Column conditions, 100% A for 30 min, followed by a linear gradient to 40% B in 48 min and then to 100% B in 2 min; flow rate: 5 ml/min; fractions were collected every 2 min. (2) Semi-preparative reversed phase (methacrylic) Amberchrom (10×250 mm, 250 Å, 35 μm, TosoHaas, Montgomeryville, PA). Column conditions, 100% A for 30 min, followed by a linear gradient to 30% B in 45 min and then to 100% B in 25 min; flow rate: 2 ml/min; fractions were collected every 2 min. (3) Confirmation of isolate purity was performed on an analytical Symmetry C_{18} (4.6×250 mm, 100 Å, 5 μm, Waters Associates, Milford, MA). Column conditions, 100% A for 10 min, followed by a linear gradient to 60% B in 60 min and then to 100% B in 10 min; flow rate: 0.5 ml/min. Peaks were collected manually. Co-elution of synthetic and isolate was completed as in 3 on a Gilson HPLC system with variable wavelength detector (214 nm; detector range: 4 Aufs). With respect to the second *Tenebrio* paralysin, purification, and identification were performed with the same order of columns as above, except for the addition of an analytical Symmetry C_8 (4.6×250 mm, 100 Å, 5 μm, Waters Associates, Milford, MA) after the second step. Column conditions, 100% A for 10 min, followed by a linear gradient to 40% B in 60 min and then to 100% B in 20 min; flow rate: 1 ml/min. Peaks were collected manually. Variations on other column conditions: Column 2 variation, linear gradient to 50% B in 75 min and then to 100% B in 20 min. Column 3 and co-elution variation, linear gradient to 15% B in 5 min, followed by an increase to 35% B in 60 min and then to 100% B in 20 min; flow rate: 1 ml/min. Aliquots of individual fractions, based on 1 (first paralysin) and 2 (second paralysin) larval equivalents, were monitored using the fly toxicity assay.

Toxicity bioassays in N. bullata and T. molitor. Ten flies from each test group were housed in clear plastic cages with ventilation and containing a water–sugar solution for nourishment. They were anaesthetised with CO_2 gas prior to injection. Synthetics or dried aliquots of HPLC-fractions were dried, dissolved in 10 μl distilled water, and subsequently injected with a 100 μl Hamilton syringe into 2- to 4-day-old adult *N. bullata*, whereas controls received 10 μl of distilled water (pH∼6.4). Effects were scored subjectively, 1 and 18 h after injection. The categorical observations ranged from dead, paralysed, reduced locomotion, inability to fly, and to no effect at all. Using a stereomicroscope, a differentiation between the states of paralysed and dead for the affected flies could be determined. Observations were made with adherence to the key along with stimulation of the flies via agitation of the cage and by visual comparison to the control group. Flies that were dead, paralysed or unable to walk (reduced locomotion) were taken into account as to measure the toxic effect. A definitive lethal effect was considered to be a paralysed or dead fly at 18 h post-injection. The *T. molitor* toxicity bioassay is similar to the fly assay. Variations on the test included a lower injection volume of 6 μl and injection into the thorax with a pulled, 1.4–1.75 mm diameter glass capillary (Vertical Pipette Puller 700C, David Kopf Instruments, USA). The beetles were kept in covered petri dish containing bran. Twenty-four hours after injection of the synthetic analogues, the beetles were observed objectively, using categories ranging from dead, paralysed, reduced locomotion to mobile.

MS analysis of purified, paralytic toxins. Nanoflow electrospray ionisation (ESI) double quadrupole (Qq) orthogonal acceleration (oa) time of flight (TOF) tandem mass spectrometry (MS/MS) was performed on a Q-TOF system (Micromass, UK). One microlitre of acetonitrile/water/formic acid (50:49:1, v/v/v) containing a small aliquot of the isolated sample or approximately 5 ng of synthetic was loaded in a gold coated capillary (Type A nanoflow needle, Micromass, UK) with the needle voltage set at 900 V and cone voltage at 25 V. The sample was sprayed at a flow rate of 30 nl/min, giving extended analysis time for acquisition of an MS spectrum along with several MS/MS spectra. During MS/MS, fragment ions are generated from a selected precursor ion by collision-induced dissociation (CID) in argon gas. Since not all selected ions fragment with the same efficiency, the collision energy is typically varied between 15 and 30 V, so the parent ion is fragmented into a satisfying number of different daughter ions.

NMR of purified, paralytic toxins. For the structural identification of the paralysins, the ^1H NMR spectra were recorded in D_2O on either a Varian Unity 600 apparatus at 600 MHz, a Bruker Avance DRX-500 instrument equipped with a z-gradient coil at 500 MHz or a Bruker Avance TM-300 at 300 MHz. Supplementary data were obtained for Paralysin IV based on two-dimensional spectra, ^1H correlated spectroscopy ($^1H/^1H$ COSY). This was performed on the 500 MHz NMR. The COSY experiment was a z-gradient-enhanced version (PFG-COSY).

X-ray diffraction. X-ray diffraction measurements were collected on a Philips PW1050 automated Bragg–Brentano diffractometer working in step-scanning mode and equipped with a proportional counter, using Ni-filtered Cu Kα radiation. The samples were powdered in an agate mortar and placed in a flat cavity mount that was not rotated during the experiment. The range of measurement of 2θ was from 5° to 50°, scan step of 0.02°, and the time of counting in every step was 1 s. Data were obtained using fixed divergence and scatter slits (1°) and a receiving slit of 0.02 mm. Si (NIST SRM640b) was used as an external standard to calibrate the instrument. The X-ray source was operated at 40 kV and 35 mA. The mean temperature of the measurement was 22 °C. Data analysis was performed using the program, Powder X [4].

Synthetic paralysins. L-Trp, THCA, and other amino acids were purchased from Sigma.

Results

Activity of larval T. molitor crude eluates in the fly toxicity bioassay

Injection of one larval equivalent from the 0–30% CH_3CN fraction as eluted from the Megabond Elute C_{18} cartridge was found to induce a lethal response within 1 h. At 10 times this concentration, the 30–60% and the 60–90% CH_3CN fractions were still devoid of any paralytic activity. Thus, further HPLC purification of the 0–30% CH_3CN fraction was performed in order to identify its toxic factor(s).

Isolation and identification of the first Tenebrio paralysin

After processing the 30% ACN fraction on the Delta-Pak C_{18} column, several fractions were found to contain toxic activity (Fig. 1A). The fractions eluting at 60–

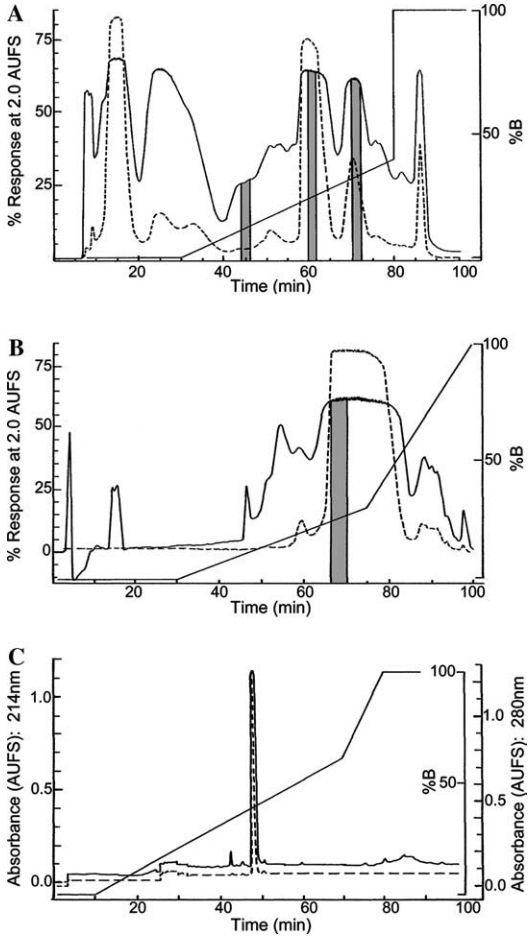


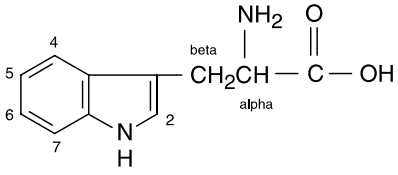
Fig. 1. HPLC chromatograms of the purification of the first *Tenebrio* paralysin. The 30% CH_3CN fraction on the first (A) Delta-Pak C_{18} column; the 60–62 min fraction from the first on the second (B) reversed phase Amberchrom column and final purification step of the 60–70 min fractions from the second on the third (C) Symmetry C_{18} column. Shaded areas represent regions of toxic activity. Absorbance: (——) 214 nm; (- - - -) 280 nm.

62 min were applied onto a reversed phase Amberchrom column where the toxicity eluted in fractions at 66–70 min (Fig. 1B). Further analysis of 2 larval equivalents of the 66–70 min fraction on an analytical Symmetry C_{18} yielded a single optically homogeneous peak eluting at 48 min (Fig. 1C), indicating that the fraction might be pure. This was confirmed by Q-TOF-MS showing an ion at m/z 204.9 $[M+H]^+$, which corresponds to a molecular mass of 203.9 Da. ^{1}H NMR analysis (Table 1) indicated a system of four aromatic hydrogen atoms (2 doublets and 2 triplets) having similar coupling constants, which is indicative of an indole structure, as found in tryptophan. Also, an ABX system was present at 4.06, 3.50, and 3.31 ppm, which could be attributed to the alpha and beta protons of this amino acid. The remaining singlet corresponds to H-2 of the indole ring. Comparison with the ^{1}H NMR spectrum of synthetic L-Trp showed almost identical data (Table 1). Fig. 2 shows the ESI induced MS/MS spectra performed on the parent ion of both the isolate and the synthetic sample. Again, the same fragments were produced, m/z 188, 170, 159, 146, 144, and 118 with the peak of greatest intensity at m/z 146. Finally, synthetic Trp perfectly co-eluted with the natural compound (48 min) on an analytical Symmetry C_{18} and it displayed the expected toxicity in the bioassay (data not shown).

Isolation and identification of the second Tenebrio paralysin

Another set of active fractions from the initial HPLC step, eluting at 70–72 min (Fig. 1A), was also applied onto a reversed phase Amberchrom column where the toxicity eluted over a period of 84–100 min (Fig. 3A). A Symmetry C_8 column resolved the toxic sample into one peak at 59 min (Fig. 3B) and subsequently, a Symmetry

Table 1
^{1}H NMR data of paralysin III:Trp (isolate and synthetic, 600 and 500 MHz, respectively)



Isolate					Synthetic				
	δ	*M*	*J*	*J*		δ	*M*	*J*	*J*
H4	7.75	d	7.8		H4	7.70	d	7.9	
H5	7.21	t	7.2		H5	7.18	t	7.3	
H6	7.29	t	7.8		H6	7.23	t	7.2	
H7	7.55	d	7.8		H7	7.50	d	8.1	
H2	7.32	s			H2	7.28	s		
αH	4.06	dd	8.4	4.8	αH	4.02	dd	8.1	4.8
βH_2	3.31	dd	15.6	8.4	βH_2	3.27	dd	15.4	8.1
	3.50	dd	15.0	4.8		3.45	dd	15.2	4.8

Note. Chemical shifts (δ) are reported in ppm, coupling constants (J) in Hz; M, multiplicity; t, triplet; d, doublet; dd, double doublet; and s, singlet.

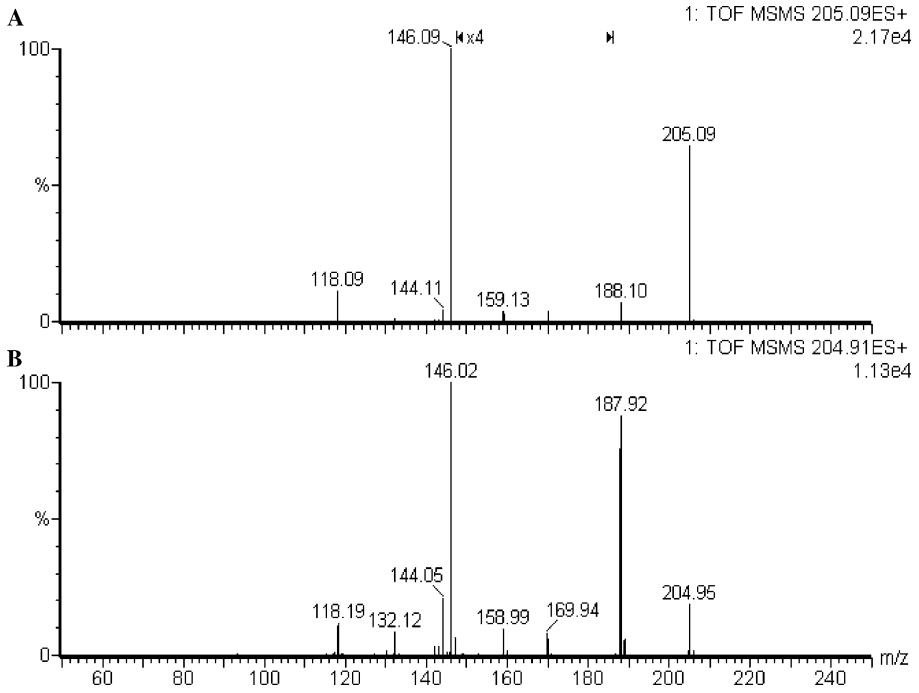


Fig. 2. The ESI CID MS/MS spectrum of the charged ion at m/z 205 $[M+H]^+$ of (A) synthetic Trp; (B) purified isolate. Ordinates are % relative intensity to peak of highest ion count; both abscissae have identical m/z ranges (50–250).

C_{18} yielded a spectrophotometrically pure peak at 46 min (Fig. 3C). Q-TOF-MS indicated that this eluate appeared to be homogeneous, containing an ion at m/z 217.09 $[M+H]^+$, corresponding to a molecular mass of 216.09 Da. ^1H NMR analysis (Table 2) of the purified compound indicated similar chemical shifts and coupling constants as in L-Trp. However, the spectrum of the second *Tenebrio* paralysin differed from that of the first one by the absence of the singlet at 7.32 ppm, attributable to H-2, and by the presence of an AB system at 4.47 ppm (H_2-1). Moreover, $^1H/^1H$ COSY completely confirmed these relationships. Given this information and the mass determined by Q-TOF-MS, it was possible to deduce that the second *Tenebrio* paralysin is THCA. This was confirmed by biochemical comparisons of the natural and synthetic compound. Fig. 4 shows the ESI fragmentation of the parent ion of both the isolate and synthetic material, displaying an identical base peak at m/z 144 and other peaks at m/z 171, 145, 143, 130, 117, 103, and 74. In addition, the ^1H and $^1H/^1H$ COSY NMR spectra of, respectively, the natural compound and synthetic THCA display nearly identical values (Table 2). Finally, synthetic THCA completely co-eluted with the natural compound (46 min) on an analytical Symmetry C_{18} and demonstrated a paralytic effect in the bioassay.

Toxicity displayed by Trp

Trp has limited solubility in water but in an acidic solution, such as the HPLC solvents used in this isolation, its solubility can increase. Thus, synthetic Trp was dissolved in aqueous 0.1% TFA (1 mg/ml) and subsequently dried prior to assaying. The gel-like sample could then be dissolved in distilled water and its ^1H NMR spectrum as well as its MS–MS fragmentation spectrum was identical to that of crystalline L-Trp. The X-ray diffraction (Fig. 5) showed that the gel-like form was amorphous, as its diffraction pattern showed one very broad peak rather than the regular characteristic "finger print" of the crystalline form. Once in an amorphous state, Trp needs less energy to go into solution due to the loss of its crystal lattice structure. This enables Trp to attain its soluble state at a faster rate, thereby facilitating the dissolving process. Therefore, one can conclude that this form of solvating augments the deliverable concentration for injection without jeopardising the real toxic effect. Thoracic injection of L-Trp into 2 to 4-day-old *N. bullata* induced a paralytic to toxic response depending on the dose, whereas D-Trp appeared to be not as potent. This appears to indicate a stereo-specific effect towards L-Trp. Other amino acids having various water affinities, glutamine and histidine (both high affinities), alanine (moderate), and valine (low), were acidified, dried, and rediluted in the same fashion as Trp. At a 200 μg dose, none of the amino acids induced a toxic effect in the fly toxicity bioassay. In adult *T. molitor* the observations were limited to 24 h post-injection, because instant paralysis is difficult to measure in beetles. Compared to the flesh fly, L-Trp is toxic but less potent in the adult beetle. At a dose of 100 μg, one-thirds of the beetles were dead or paralysed,

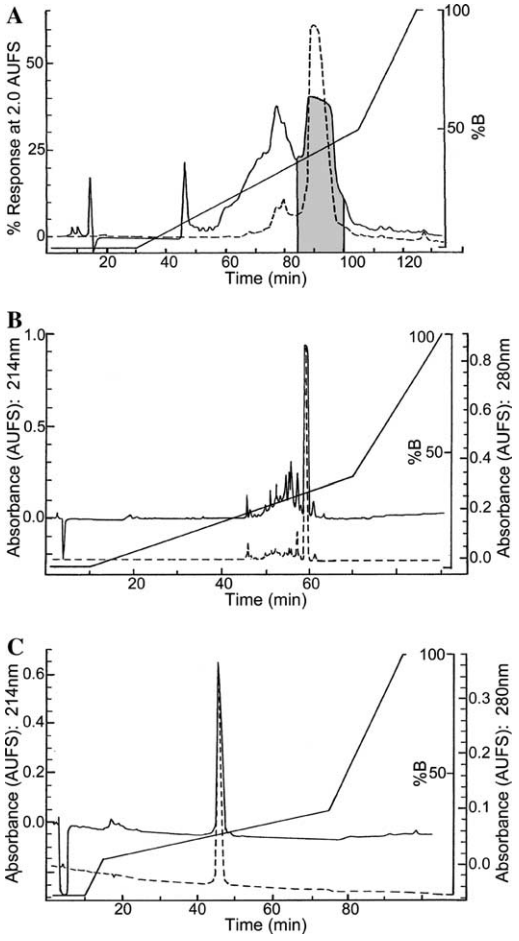


Fig. 3. HPLC chromatograms of the purification of the second *Tenebrio* paralysin. The 70–72 min fraction of Fig. 1A on the second (A) reversed phase Amberchrom column; the 84–100 min fractions from the second on the third (B) Symmetry C_8 column and the peak eluting at 59 min from the third on the final (C) Symmetry C_{18} column. Shaded areas represent regions of toxic activity. Absorbance: (——) 214 nm; (- - - -) 280 nm.

whereas a mortal effect was observed in half of the flesh flies using this dose.

Toxicity displayed by THCA

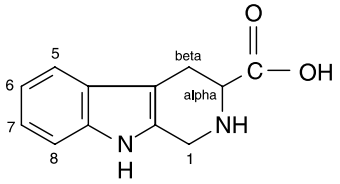
Unfortunately, synthetic THCA has a low solubility in water. Other solvents of varying pH and polarity, such as NaOH, NH_3, HCl, ethanol, Tween or DMSO could not increase solubility without augmenting the sample's toxicity. This has left the option to use the supernatant of a mixture, initially prepared for 200 μg THCA/10 μl distilled water. Initial results show that no immediate effect was recorded in the adult flesh fly, but 18 h later, two-thirds of the flies are paralysed or dead. As for the beetle, three-quarters are displaying a lethal response 24 h post-injection. Again, control injections with distilled water are not toxic.

Discussion

Guided by a toxicity assay, we have successfully isolated and identified two new paralytic substances, Trp and THCA, endogenous to the larval mealworm. Both compounds share a common indole structure.

Physiologically, Trp is either a substrate for protein synthesis or is metabolised to a number of biologically important substances. It is now well established that in mammals, 90% of Trp is converted to nicotinamide adenine dinucleotide (NAD) via the kynurenine pathway by oxidative cleavage of the indole nucleus between carbons C-2 and C-3 [5,6]. In insects only one catabolic Trp pathway exists. Initially, it is similar to the mammalian's, however, it is blocked at the oxidation of 3-hydroxyanthranilic acid, thereby negating the formation

Table 2
^{1}H NMR data of paralysin IV:THCA (isolate and synthetic, 500 and 300 MHz, respectively)



Isolate					Synthetic				
	δ	*M*	*J*	*J*		δ	*M*	*J*	*J*
H5	7.58	d	7.8		H5	7.51	d	7.8	
H6	7.13	t	7.4		H6	7.06	t	7.3	
H7	7.21	t	7.6		H7	7.14	t	7.4	
H8	7.44	d	8.1		H8	7.36	d	8.0	
H_2-1	4.47	AB	15.7		H_2-1	4.34	AB	16.3	
αH	4.05	dd	10.7	5.3	αH	3.90	dd	10.5	5.6
βH_2	3.02	dd	16.0	11.0	βH_2	2.92	dd	16.1	10.3
	3.38	dd	16.4	5.0		3.28	dd	16.0	5.2

Note. Chemical shifts (δ) are reported in ppm, coupling constants (*J*) in Hz; M, multiplicity; t, triplet; d, doublet; dd, double doublet; and AB, AB system.

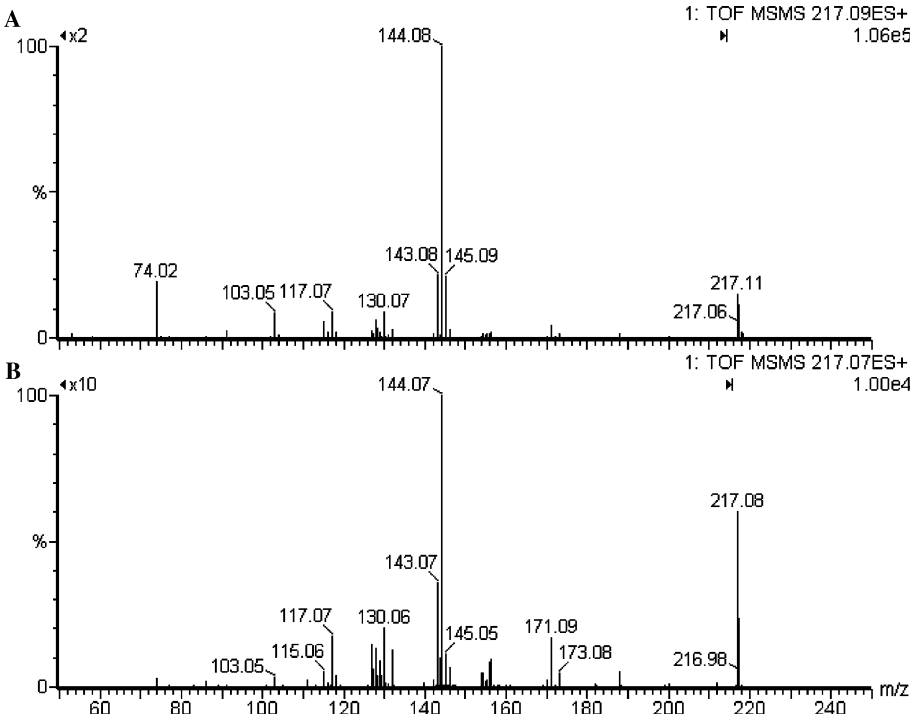


Fig. 4. The ESI CID MS/MS spectrum of the charged ion at m/z 217 $[M+H]^+$ of: (A) synthetic THCA; (B) purified isolate. Ordinates are % relative intensity to peak of highest ion count; both abscissas have identical m/z ranges (50–250).

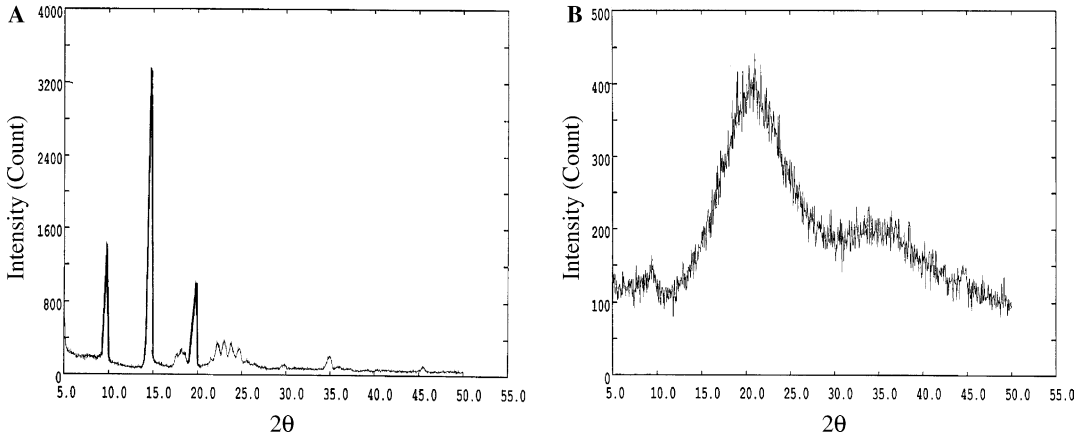


Fig. 5. X-ray diffraction pattern of crystalline (left); acidified/dried (right) L-Trp.

of NAD. Rather, a group of pigments, known as the ommochromes, are formed, rendering its common name, the ommochrome pathway. These pigments contribute to the deep tinge of insect eyes and the brilliant colouration of many species [7]. These metabolites, common to both classes, are generally referred to as the kynurenines. A major point of interest is that 3HK, recently isolated from larval *N. bullata* based on its paralytic activity [2], is a kynurenine metabolite of Trp. With respect to insects, mutant Trp metabolising organisms have given scientists the opportunity to study the effects of excess Trp in vivo. In the mutant strains, snow of *Apis mellifera* [8] and vermilion of *Drosophila melanogaster* [9], the conversion of Trp to formylkynurenine is blocked, thus leading to an excess of unbound Trp and an absence of the subsequent metabolites of the ommochrome pathway. This excess of Trp results in a poor performance of signal behaviour (dance), in rhythm and an overall reduction in the number of dancing bees. This mutation also reduces the excitability of neuromuscular activity both at the larval and adult stage. Exogenously applied Trp to the wild strain bees results in all the characteristics observed in the snow mutants. Evidence of neurodegeneration [10]

has been observed in vermilion flies. Some scientific research and epidemiological studies directly focusing on Trp toxicity in mammals exist as well [11]. Intraperitoneal Trp administration (~40–1201 mg/rat) to rats produces an analgesic response and at higher levels, a significant decrease in pain sensitivity to a hotplate test [12]. Possibly, the result could be attributed to a diminished motor neuron response rather than a reduction in pain detection. Metodiewa [13] has proposed that Trp can cause cellular injury leading to neurological disorders given that during its oxidative degradation process in vitro, Trp acts as a generator of reactive oxygen species: peroxides, superoxide, and peroxy radicals. One can speculate that Trp is responsible for altering normal nerve function in mammals and insects. Its high levels followed by subsequent metabolism during metamorphosis in insects [7] may be employed for immobilisation of the organism and neuronal tissue remodelling while its metabolism into ommochromes is a sparing and functional path for insect survival.

In this paper, we report for the first time, the extraction of the β-carboline, THCA from an insect and, moreover, its in vitro (neuro) toxic properties in this class of animals. THCA is a member of the β-carbolines, a tricyclic, indole alkaloid family. β-Carbolines are categorised with respect to the saturation of their N-containing, six-membered ring. Unsaturated members are referred as aromatic β-carbolines, whereas the partially or completely saturated ones are known, respectively, as dihydro-β-carbolines (DHBCs) and tetrahydro-β-carbolines (THBCs). THCA is a modified THBC due to the presence of a carboxylic acid group on the αC of the saturated ring.

It has been known for some time that β-carbolines, in particular the aromatics, are abundant in plants and easily extracted for the common use of their psychoactive and therapeutic properties [14,15]. Yet, only in 1975, were Okuda et al. [16] the first to isolate and identify THCA from a natural source. They extracted 53 mg of THCA from 12 kg of *Aleurites fordii* seeds. Evidence for the natural occurrence of β-carbolines in animals also exists. THBC, in the form of 6-hydroxy-MTCA, has been detected in rat liver, kidney, plasma, urine, and faeces [17] and 6-methoxy-THBC has been measured in the pineal gland of the chicken and cock [18]. THBC has been quantified in rat brain and adrenal glands along with other analogues [19]. MTBC has been detected in the urine [20] and aromatic β-carbolines in the blood [21] of healthy men and women. However, β-carboline de novo synthesis remains in contention today, since many fresh, processed, cooked, and preserved foods, alcoholic beverages, and cigarette smoke condensates contain β-carbolines, including THCA and MTCA, which can be a significant exogenous source [22,23]. In any case, it is apparent that β-carbolines are part of living tissue and their biological effects are a realistic consequence. Relevant to the issue of (neuro) toxicity is the convulsant behaviour related to the binding of β-carbolines to the benzodiazepine (BDZ) site of GABA receptors. Harman, an aromatic beta-carboline, induces convulsions while harmaline, a DHBC, causes tremors in mice [24]. The harmaline-induced tremor is prevented by the BDZ ligand diazepam [25]. β-Carbolines are reported as inverse-agonist ligands for BDZ receptors [26]. Finally, Malgrange et al. [27] have observed apoptotic cell death in cerebellar granule neuron culture when exposed to modified aromatic β-carbolines, while GABA and benzodiazepine ligands such as diazepam protect this culture from the β-carboline induced toxicity.

All paralysins have a high level of activity during metamorphosis that quickly disappears before maturity, as seen in the toxic activity of whole body extracts from developing *N. bullata* and *T. molitor*. Furthermore, this phenomenon appears to be universal in the class of insects and its function may involve the immobilisation of the insect for its physiological transformation and possibly in the selective breakdown of neuronal tissue [1,2,28]. In the adult stage the levels of the paralysins decrease significantly, a way to avoid toxic effects in this stage. A practical formation of pigments or sclerotizing agents appears to be the outcome. Both in mammals and insects, Trp is the precursor to kynurenine formations that involve 3HK and it may be involved in the formation of THCA [5,6,29]. Similar to insects, Trp and kynurenine levels increase prior to mammalian birth, then dropping and remaining at a constant level in healthy mammals. When levels of any of these paralysins are not regulated, neurotoxic responses occur. No data are as yet available in the literature on the levels of β-carboline during animal development. Given the high levels of activity of Trp and β-carboline during insect metamorphosis, it is imperative in the future to further define the physiological significance of the present discoveries by determining free Trp and β-carboline levels during insect development and ultimately their mode of action.

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