than the compounds mentioned elsewhere in this chapter.

Conclusion

TLC has many advantages for the analysis of herbal products, especially phytopharmaceuticals, for the identification of plants and the quantification of certain marker substances. Planar chromatography has advantages because it allows a parallel evaluation and comparison of multiple samples. In addition, various chromatographic separation systems can be combined with a multitude of specific and non-specific derivatizing agents. Even in samples having complex matrixes such as the pharmaceutical preparations of extracts of plants, sample preparation can be kept simple because of the use of the stationary phase for only one analysis. Unlike column chromatography, contamination of the chromatographic system by carryover cannot occur. In many instances the chemical composition of the herb is not completely known and for many plants, there are often no established methods of analysis available so that a rapid screening technique like TLC is very valuable. Constituents of herbals that belong to very different classes of chemical compounds can often create difficulties in detection, but with this in mind, TLC can offer many advantages.

See Colour Plates 105, 106.

See also: **III/Alkaloids:** High Speed Counter Current Chromatography; Liquid Chromatography; Thin-Layer (Planar) Chromatography. **Citrus Oils: Liquid Chromatography. Essential Oils:** Distillation; Gas Chromatography; Thin-Layer (Planar) Chromatography. **Pigments:** Liquid Chromatography; Thin-Layer (Planar) Chromatography. **Terpenoids: Liquid Chromatography. Appendix 17/Thin-Layer (Planar) Chromatography: Detection.**

Further Reading

Anonymous (1998) Camag bibliography service. CD ROM. Muttenz, Switzerland: Camag.

Baerheim Svendsen A and Verpoorte R (1983) *Chromatography of Alkaloids. Part A: Thin Layer Chromatography*. Elsevier: Amsterdam.

Bruneton J (1999) *Pharmacognosy*, (*Phytochemistry Medicinal Plants*), 2nd edn. Paris: Tec and Doc.

Fried B and Sherma J (1994) *Thin Layer Chromatography: Technique and Applications*. New York: Marcel Dekker.

Fried B and Sherma J (1996) *Practical Thin Layer Chromatography*, pp. 31–47. Boca Raton: CRC Press.

Geiss F (1987) *Fundamentals of Thin Layer Chromatography*. Heidelberg: Hüthig.

Harbone JB (1973) *Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis*. London: Chapman and Hall.

Jork H, Funk W, Fisher W and Wimmer H (1990) *Thin Layer Chromatography Reagents and Detection Methods*, Vol 1a. Weinheim: VCH.

Randerath K (1967) *Dünnschicht Chromatographie*, 2nd edn. Berlin: Springer Verlag.

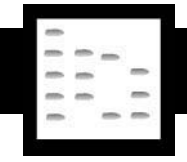
Stahl E (1967) *Dünnschicht Chromatographie*, 2nd edn. Berlin: Springer Verlag.

Touchstone J (1992) *Practical Thin Layer Chromatography*, 3rd edn. London: John Wiley.

Wagner K, Bladt S and Zgainski EM (1966) *Plant Drug Analysis*. Berlin: Springer Verlag.

Wichtl M (1994) *Herbal Drugs and Pharmaceuticals*. Boca Raton: CRC Press.

NEUROTOXINS: CHROMATOGRAPHY



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Chromatography has had a major impact on the discovery and detection of potent, naturally occurring neurotoxins. The neurotoxins discussed in this article were selected because they significantly impact on human health as a result of intoxications from bites and stings or the consumption of contaminated food and water. Many of these toxins target receptors that have implications for the development of potential therapeutic agents. In the neurotoxin topics that have been highlighted here, the role of chromatography in toxin discovery, purification and analysis is emphasized.

Neurotoxins from Marine and Freshwater Algae

It was only in the latter part of the 20th century that scientists appreciated that certain species of microalgae can cause sporadic toxic events that can lead to serious illness, with occasional deaths, in humans as well as farmed and domestic animals. When high populations of toxin-producing microalgae occur

Table 1 Representative neurotoxins that are found in microalgae and marine food

Toxin	*Toxin potency, LD_{50} μg kg^{-1}*	*Poisoning syndrome*	*Food type*	*Typical LC method*
Saxitoxin	3	Paralytic shellfish poisoning (PSP)	Shellfish, freshwaters	LC-FL
Azaspiracids	140–200	Azaspiracid poisoning (AZP)	Shellfish	LC-MS
Anatoxin-a	200	'Very fast death factor'	Freshwaters	LC-UV, LC-FL
Anatoxin-a(s)	20	–	Freshwaters	LC-MS
Tetrodotoxin	8	Puffer fish poisoning	Puffer fish, crabs	LC-FL
Brevetoxin-a	95	Neurological shellfish poisoning (NSP)	Shellfish	LC-UV
Ciguatoxin	0.45	Ciguatera	Finfish	LC-MS

LD_{50}, lethal dose at 50% mortality, for 14–20 g mouse, when delivered intraperitoneally (IP).

they are termed harmful algal blooms (HABs) and there is compelling evidence to suggest that there is a global increase in the frequency of such events. Molluscs are the marine animals most susceptible to this toxicity, especially the filter-feeding varieties such as mussels, clams, scallops and oysters, which accumulate these toxins to hazardous concentrations. However, potent neurotoxins can also occur in finfish, toxins can accumulate in food plants, while skin absorption of toxins from bacteria is also possible. The comparative potencies of the main neurotoxins that are found in microalgae and marine food are shown in **Table 1**. Their high toxicities, together with the worldwide occurrence of these toxins, have led to a requirement for sensitive analytical methods for their determination. Ciguatoxin is a lipid-soluble toxin and occurs in the flesh of finfish in tropical and subtropical waters. It exerts its effect by activating voltage-dependent sodium channels, producing both gastrointestinal and neurological symptoms with occasional fatalities. Other neurotoxins that act on sodium channels include (1) tetrodotoxin, first discovered in puffer fish, (2) saxitoxin, responsible for paralytic shellfish poisoning (PSP) and (3) brevetoxins, responsible for neurological shellfish poisoning (NSP). A new human toxic syndrome, azaspiracid shellfish poisoning (AZP), which is caused by the consumption of mussels, has recently been identified in Europe. Cyanobacteria (blue-green algae) can produce both hepatotoxins and neurotoxins and represent a serious hazard because of their contamination of freshwater lakes and reservoirs used by animals and for human consumption. Anatoxin-a is the most common neurotoxin in freshwaters but PSP toxins have also been reported. Chromatography continues to play an important role, not only in the discovery of neurotoxins, but also in the development of analytical methods that are used for their quantitative determination for regulatory control and for forensic investigation of intoxications. Highly sensitive chromatographic detection methods, especially using fluorescence (liquid chromatography–fluorescence, LC-FL) and mass spectrometry (liquid chromatography–mass spectrometry, LC-MS), have been particularly important and have been critical for the identification of the microalgae responsible for producing specific neurotoxins.

Neurotoxins from Cyanobacteria (Blue-Green Algae)

There have been reports since the 19th century of animal mortalities associated with drinking water contaminated by cyanobacteria. These phenomena have occurred throughout the world and have been attributed to both hepatotoxic microcystins and to neurotoxins which are produced by some species of cyanobacteria. There are three classes of neurotoxins that are commonly found in cyanobacteria: (1) saxitoxin and analogues, (2) anatoxin-a(s), (3) anatoxin-a and analogues. These compounds are shown in **Figures 1–3**.

Saxitoxin and analogues The most spectacular neurotoxic event due to cyanobacteria occurred in Australia in 1991 when a toxic bloom in the Darling river resulted in the deaths of 1600 sheep and other animals. These intoxications were attributed mainly to neurotoxins belonging to the saxitoxin group, which were previously identified as PSP toxins. These toxins are discussed elsewhere. The investigation of

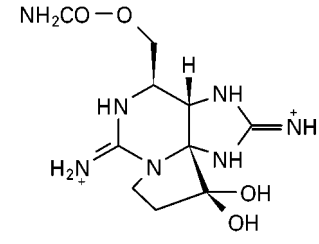

Figure 1 Saxitoxin.

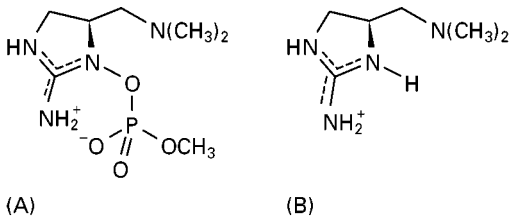

Figure 2 (A) Anatoxin-a(s), m/z 253.1; (B) fragment ion, m/z 143.1.

these events relied heavily on the application of the fluorimetric high performance liquid chromatography (HPLC) method (LC-FL) for saxitoxins, developed by Oshima. This method uses paired ion reagents with three sets of isocratic reversed-phase chromatographic conditions to separate 18 analogues, which are detected by post-column oxidation and form highly fluorescent products. In addition to the PSP toxins that have previously been identified in shellfish, a number of new PSP analogues have recently been isolated from cyanobacteria.

Anatoxin-a(s) Anatoxin-a(s) is a unique organophosphate toxin and it is a potent cholinesterase inhibitor. This neurotoxin has been identified in cyanobacteria in North America and in Europe where it has been fatal to dogs and birds. However, this toxin is difficult to detect as the chromatographic sensitivity using ultraviolet light (LC-UV) is very poor and it is probably more widespread in nature than has so far been discovered. Anatoxin-a(s) is unstable, particularly at slightly basic pH, but has been determined using fast atom bombardment–mass spectrometry (FAB-MS). However, even a 'soft' ionization technique such as electrospray LC-MS produces mainly the fragment ion at m/z 143.1 (Figure 2B), due to loss of the phosphate moiety. Fortunately, this ion is sufficiently characteristic to allow the screening of water and algae samples for the presence of anatoxin-a(s).

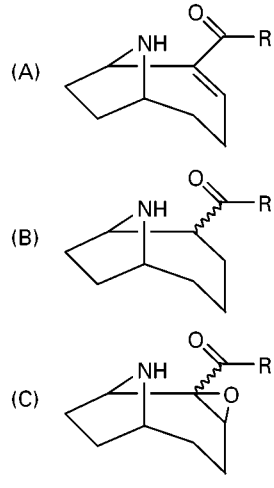

Figure 3 (A) Anatoxin-a ($R = CH_3$), homoanatoxin-a ($R = CH_2CH_3$); (B) dihydroanatoxin-a ($R = CH_3$), dihydrohomoanatoxin-a ($R = CH_2CH_3$); (C) epoxyanatoxin-a ($R = CH_3$), epoxyhomoanatoxin-a ($R = CH_2CH_3$).

Anatoxin-a and analogues Anatoxin-a (Figure 3A, $R = CH_3$) was the first cyanobacterial toxin to be structurally elucidated and it is a potent nicotinic agonist which acts as a depolarizing neuromuscular blocking agent. Typical symptoms in animals include muscle fasciculations, gasping and convulsions, with death due to respiratory arrest within minutes after drinking contaminated water. In fact, fatalities to animals, including cattle and dogs, were so rapid that before the identification of anatoxin-a this toxin was referred to as 'very fast death factor'. A related toxin, homoanatoxin-a (Figure 3A, $R = C_2H_5$), was isolated recently in Norway.

Several chromatographic methods are available for the analysis of anatoxin-a in cyanobacterial bloom material, including LC-UV and LC-MS using electrospray ionization. Derivatizations of anatoxins, followed by gas chromatography (GC) with electron capture or MS detection have also been successful. A highly sensitive LC-FL method has been developed by the authors for the determination of anatoxin-a, using derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F). This has been applied to the analysis of the anatoxins and their degradation products, the dihydro (Figure 3B) and epoxy (Figure 3C) analogues, which result from the reduction or oxidation of the alkene moiety. These degradation products are not detected by the commonly used LC-UV method as they do not have the α,β-unsaturated ketone that is present in the parent toxins.

The determination of anatoxin-a in raw waters poses greater analytical problems mainly due to the low natural concentration of this toxin. However, Harada has developed an efficient solid-phase extraction (SPE) procedure in which anatoxin-a and analogues are efficiently extracted from water using a weak cation exchange phase. After trapping the anatoxins on the SPE cartridge, which is washed with methanol–water, they are readily eluted using acidic methanol since they are basic compounds. All of these anatoxins can be derivatized by reaction with NBD-F (**Figure 4**), at room temperature for several minutes, to produce highly fluorescent products that are readily separated using isocratic reversed-phase LC-FL. A typical chromatogram showing the separation of the NBD derivatives of anatoxin-a, homoanatoxin-a and their degradation products is shown in **Figure 5**A. The detection limit for anatoxin-a

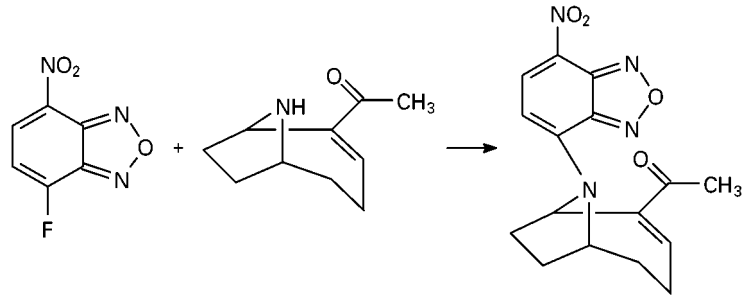

Figure 4 Reaction of anatoxin-a with NBD-F to produce a fluorescent product.

is 0.02 ng mL^{-1}, which allows this method to be applied to the routine monitoring of water supplies as well as for the forensic investigation of toxic incidents. This method was used to investigate the deaths of two dogs near Lough Derg, Ireland, two weeks after the event. Figure 5B shows a chromatogram from the investigation in which the dihydroanatoxin-a isomers are separated and are present in higher levels than anatoxin-a. Frequently, the detection of dihydroanatoxins in old samples may be the only evidence to implicate anatoxins in an intoxication event as anatoxin-a is unstable and readily decomposes at basic pH and in light.

Azaspiracid Poisoning (AZP) – A New Human Toxic Syndrome

Azaspiracid (formerly KT-3) is a marine toxin responsible for a new toxic syndrome, AZP. The first confirmed occurrence of this toxicity was in November 1995 in the Netherlands when at least eight people reported severe illness after the consumption of cultured mussels from the west coast of Ireland (Killary Harbour). Although human symptoms, which included vomiting, severe diarrhoea and stomach cramps, were similar to diarrhoetic shellfish poisoning (DSP), only insignificant levels of DSP toxins were detected using LC-FL. The isolation of the major toxin was difficult, which is typical when dealing with a complex matrix such as shellfish tissue, and relied on the use of a variety of preparative chromatographic phases, as described by Satake and co-workers. Starting with 20 kg of mussel meat, the extract from several solvent extraction procedures was first subjected to adsorption chromatography using silica, followed by gel permeation chromatography. Next, carboxymethyl (CM) and diethylaminoethyl (DEAE) weak ion exchange phases were used and the final purification again used gel permeation to give 2 mg of azaspiracid.

Azaspiracid is characterized by a trispiro assembly and an azaspiro ring moiety that is unique in nature (**Figure 6**). In 1997 azaspiracid was again responsible for a toxic incident, which occurred in Arranmore Island, Ireland, with more than 12 local human intoxications. There have been several other reported incidents, in Italy and France, and two analogues of azaspiracid have also been isolated, namely methylazaspiracid (AZ-2) and demethylazaspiracid (AZ-3). There have only been limited toxicological studies of azaspiracids. Mice administered high doses of azaspiracid died after short periods, showing neurotoxic symptoms, while morphopathological studies showed that the target organs are the liver, spleen and digestive tract.

Azaspiracids, like most other shellfish toxins, are produced by dinoflagellates but the causative organism is as yet unknown. However, unlike other types of shellfish toxicity, natural depuration of azaspiracids is very slow and toxins can persist in shellfish for as long as eight months. The development of analytical methods to determine azaspiracids in seafood is therefore a priority research topic and LC-MS has proved invaluable for the monitoring and management of toxic outbreaks. **Figure 7** shows the chromatograms obtained from a crude extract from mussels using electrospray ion-trap mass spectrometry, without using any clean-up procedure. To achieve further confirmation of toxin identity, liquid chromatography–collision-induced dissociation–mass spectrometry (LC-CID-MS), with a collision energy of 40%, gave a characteristic fragmentation due to sequential loss of water molecules, as shown in **Figure 8**.

Venoms from Snakes and Spiders

Neurotoxic Peptides

More than 90% of the snake venoms produced by mambas, cobras and tiger snakes (which all belong to the family *Elapidae*), contain small protein molecules that are responsible for a wide range of toxicological and pharmacological activities. The complex mixtures of polypeptides that are present in most snake

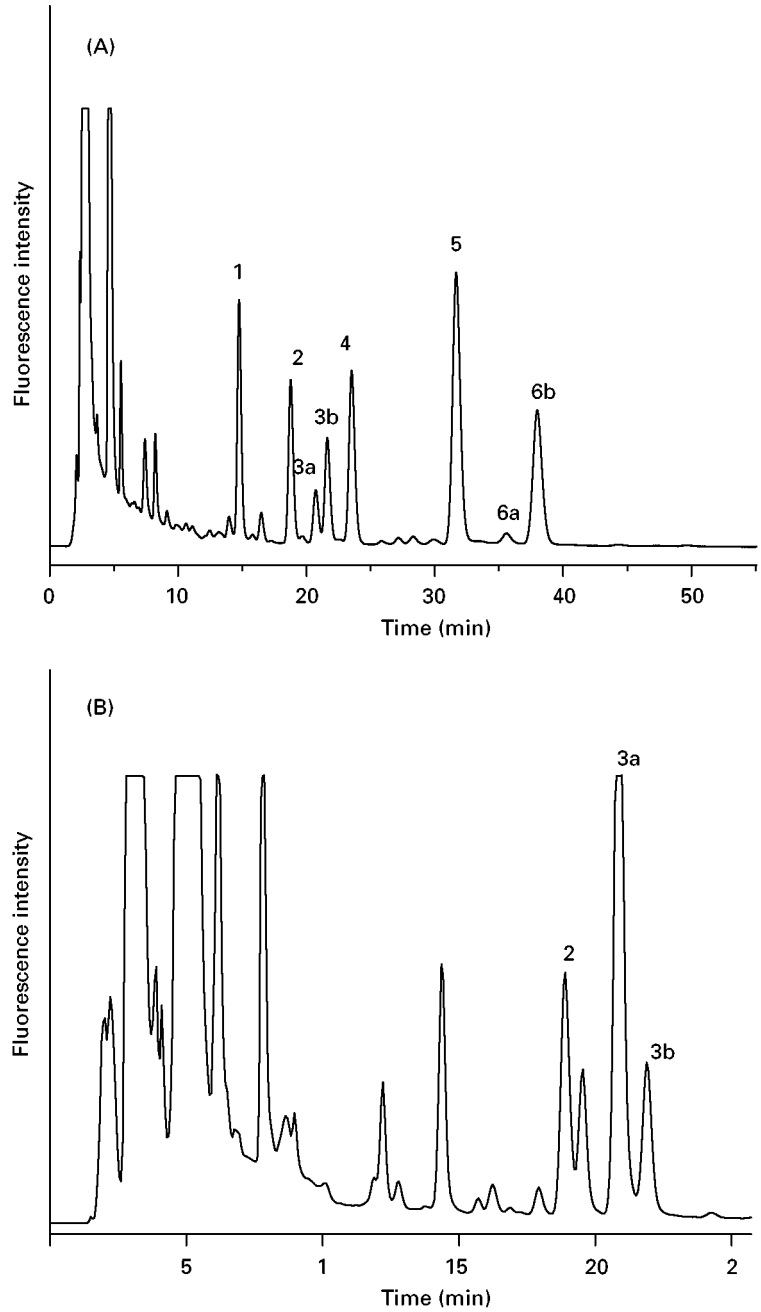

Figure 5 (A) Chromatogram from the fluorimetric HPLC analysis of anatoxin standards following derivatization with NBD-F. 1, NBD-anatoxin-a epoxide (14.7 min, 4.1 ng); 2, NBD-anatoxin-a (18.7 min, 3.5 ng); 3a, NDB-dihydroanatoxin-a isomer 1 (20.7 min, 1.2 ng); 3b, NDB-dihydroanatoxin-a isomer 2 (21.6 min, 2.5 ng); 4, NBD-homoanatoxin-a epoxide (23.5 min, 4.3 ng); 5, NBD-homoanatoxin-a (31.6 min, 8.8 ng); 6a, NBD-dihydrohomoanatoxin-a isomer 1 (35.5 min, 0.4 ng); 6b, NBD-dihydrohomoanatoxin-a isomer 2 (37.9 min, 5.2 ng). Reproduced with permission from James KJ *et al.* (1998) *Journal of Chromatography* 798: 147–157. (B) Chromatogram obtained using a water sample from Lough Derg, Ireland, showing the presence of NBD derivatized anatoxins. This water contained anatoxin-a (2.1 $\mu g\,L^{-1}$), dihydroanatoxin-a isomer 1 (49 $\mu g\,L^{-1}$) and dihydroanatoxin-a isomer 2 (1.6 $\mu g\,L^{-1}$). HPLC conditions: 5 μm Prodigy C_{18} column (250 $\times$ 3.2 mm); temperature, 35°C; mobile phase, acetonitrile–water (45 : 55, v/v); flow rate, 0.5 mL min^{-1}; fluorescence detection, λ_{ex} 470 nm, λ_{em} 530 nm.

venoms can be generally divided into neurotoxins and cardiotoxins (6000–10 000 amu), together with phospholipases A_2 (*c.* 13 000 amu) and larger enzymes. Often, the toxic effects of these peptides result from a synergistic effect of the polypeptides rather than from a high intrinsic toxicity of individual compounds, and they have attracted interest for their potential therapeutic applications.

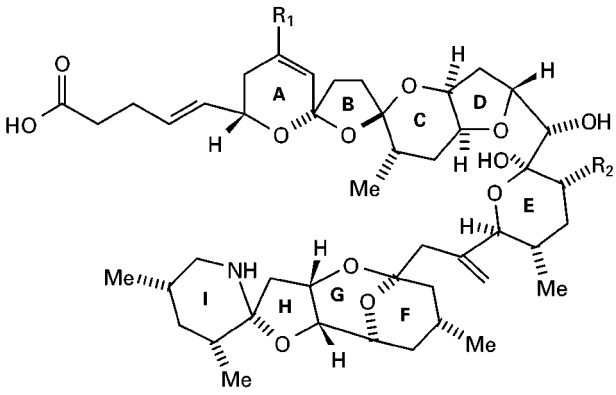

Figure 6 Azaspiracid (AZ-1, $R_1 = H$, $R_2 = Me$), methylazaspiracid (AZ-2, $R_1 = R_2 = Me$), demethylazaspiracid (AZ-3, $R_1 = R_2 = H$).

The analysis of snake venoms has traditionally relied on chromatographic separations using gel filtration and ion exchange phases, with protein size determined using methods such as sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Subsequent sequencing of the amino acids in the isolated proteins can be carried out by automated Edman degradation with a gas phase microsequencer. Using these techniques, the isolation of 28 peptides from the venom of the black mamba snake has been reported, most of which are structurally related cationic peptides, called dendrodotoxins, with similar activities. However, the application of capillary electrophoresis–electrospray ionization–mass spectrometry (CE-ESI-MS) has been shown to be a particularly effective technique for the separation and analysis of such complex mixtures of small proteins as are found in these venoms. A problem that is often encountered when separating basic peptides is the retention of a nett positive charge that leads to peak broadening due to the sorption of analytes to the negatively charged column wall. Several column wall derivatizing reagents have been reported to minimize this problem and it has been shown by Tomer and co-workers that when CE is carried out using a fused silica column derivatized with 3-aminopropyltrimethoxysilane (APS), the charge on the capillary wall becomes positive. Excess negative ions in solution drive the electroosmotic flow from a high negative potential to ground, and positively charged analyte ions are repelled by the column wall. ESI-MS is particularly useful for the analysis of large protein analytes, as these become multiply charged. The *m/z*

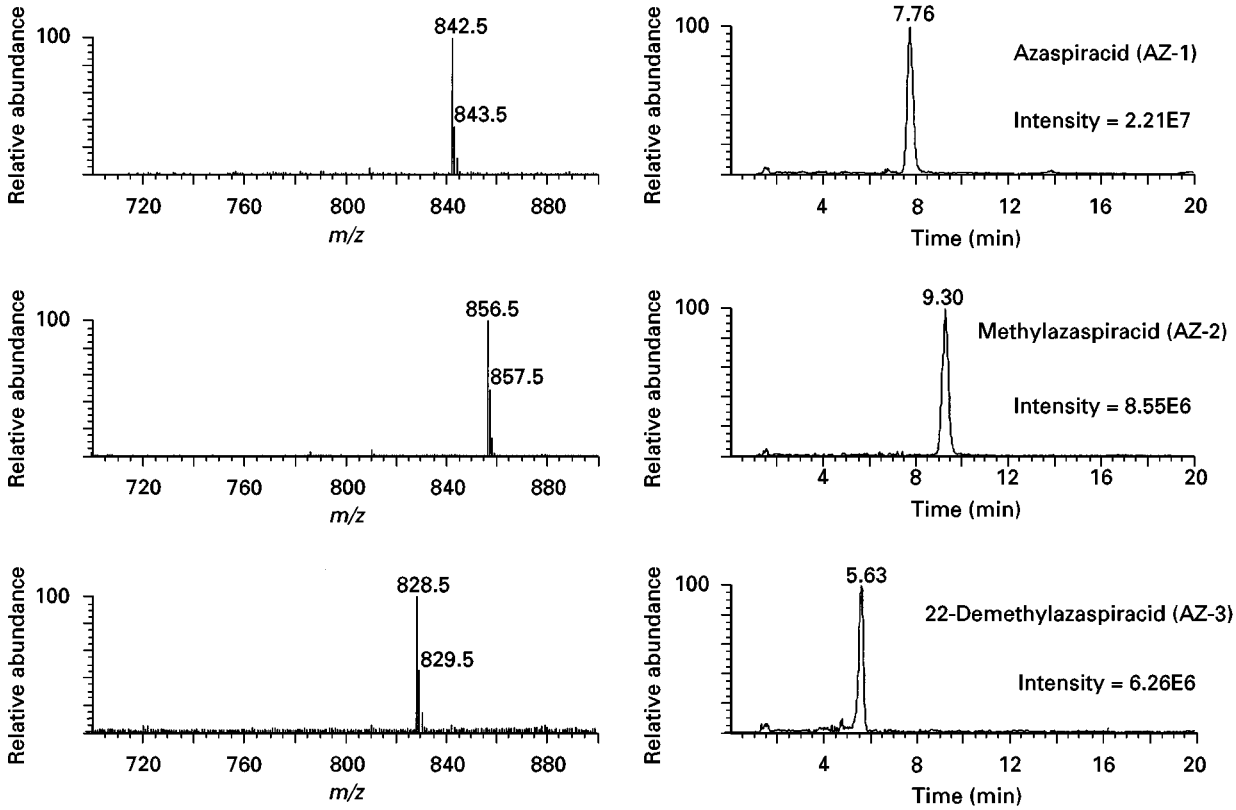

Figure 7 Electrospray LC-MS analysis of a toxic shellfish sample implicated in human intoxication. Azaspiracid (14.7 $\mu g\,g^{-1}$), methylazaspiracid (13 $\mu g\,g^{-1}$), 22-demethylazaspiracid (8 $\mu g\,g^{-1}$). Chromatographic conditions: C_{18} Luna column (5 μm, 250 × 3.2 mm, Phenomenex); 25°C; acetonitrile–water (70 : 30) containing 0.50% TFA; Flow rate 0.2 mL min^{-1}.

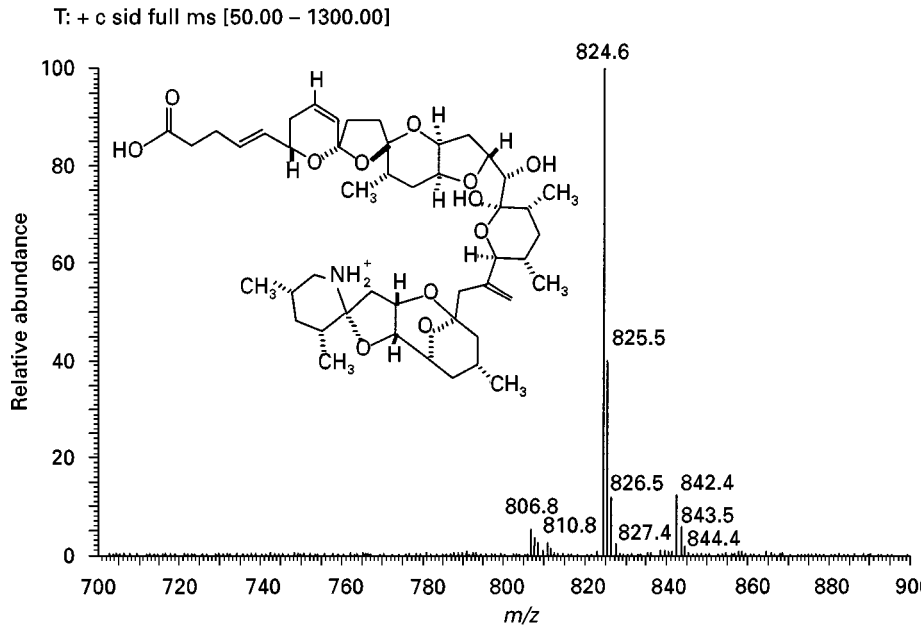

Figure 8 Mass spectrum obtained by LC-CID-MS of azaspiracid at 40% collision energy showing fragment ions for $[M + H–H_2O]^+$ and $[M + H–2H_2O]^+$ at m/z 824.5 and 806.4, respectively.

ratio (determined by the mass spectrometer) is consequently reduced, which permits the determination of higher charged large molecules using spectrometers with significantly lower mass ranges. For example, the CE-ESI-MS analysis of the black mamba snake venom showed two dominant ions in the spectrum at m/z 1020 and 1090 which were related to the $[M + 7H]^{7+}$ and $[M + 6H]^{6+}$ ions of toxin 1 (7133.5 amu), previously shown to be the predominant dendrodotoxin.

Acylpolyamine Neurotoxins from Spider Venoms

The tendency among humans to avoid contact with spiders is attributed, in part, to the ability of some species to deliver potent venoms. This venomous capability is used largely to paralyse or kill prey, particularly insects, but also affects a wide range of invertebrate and vertebrate animals. Neurotoxins are important as tools in neurochemical research and to investigate the functioning of neural receptors and ion channel modulators. Chemical studies on spider venoms have been hampered by the fact that many proteins, polypeptides and polyamines are often present in samples that are difficult to acquire in sufficient quantities. Special interest has been shown in acylpolyamines, low molecular weight toxins from spiders that antagonize specific glutamate receptors, since there are few other examples of this activity. Neural functions affected include memory and motor control and these toxins are important in studies to design potential therapeutic agents.

Acylpolyamines were first discovered in the 1980s and some examples are shown in **Figure 9**. They contain an aromatic ring connected to chains containing amide and amine moieties with some also incorporating amino acids, particularly arginine. The lengths of the polyamine chains vary considerably, as they can contain from 7 to 43 atoms, and they are linked to various phenol or indole rings. Examples of toxins with a dihydroxybenzene ring include NSTX-3 (Figure 9A-2, X = C) and JSTX-3 (Figure 9A-3, X = C).

Several chromatographic techniques have been applied to separate these complex mixtures, which can contain as many as 50 acylpolyamines in a single spider venom sample. Preparative HPLC can be used directly to separate toxins in an aqueous extract of spider venom using photodiode-array UV detection. However, tandem LC, combining UV detection with on-line fluorescence detection, following reaction with *o*-phthalaldehyde (OPA), has proved valuable for the detection of minor components. For these separations, a linear gradient of water (containing 0.1% trifluoroacetic acid), for example 5–60% acetonitrile, can be used for reversed-phase chromatography. Fractions containing active constituents typically require up to three further preparative LC steps, with both ion exchange and octadecylsilica (ODS) columns, to purify toxins to homogeneity. Bioassays of fractions to detect toxin activity are also frequently used in these studies and an example is the assay of histamine release from rat peritoneal

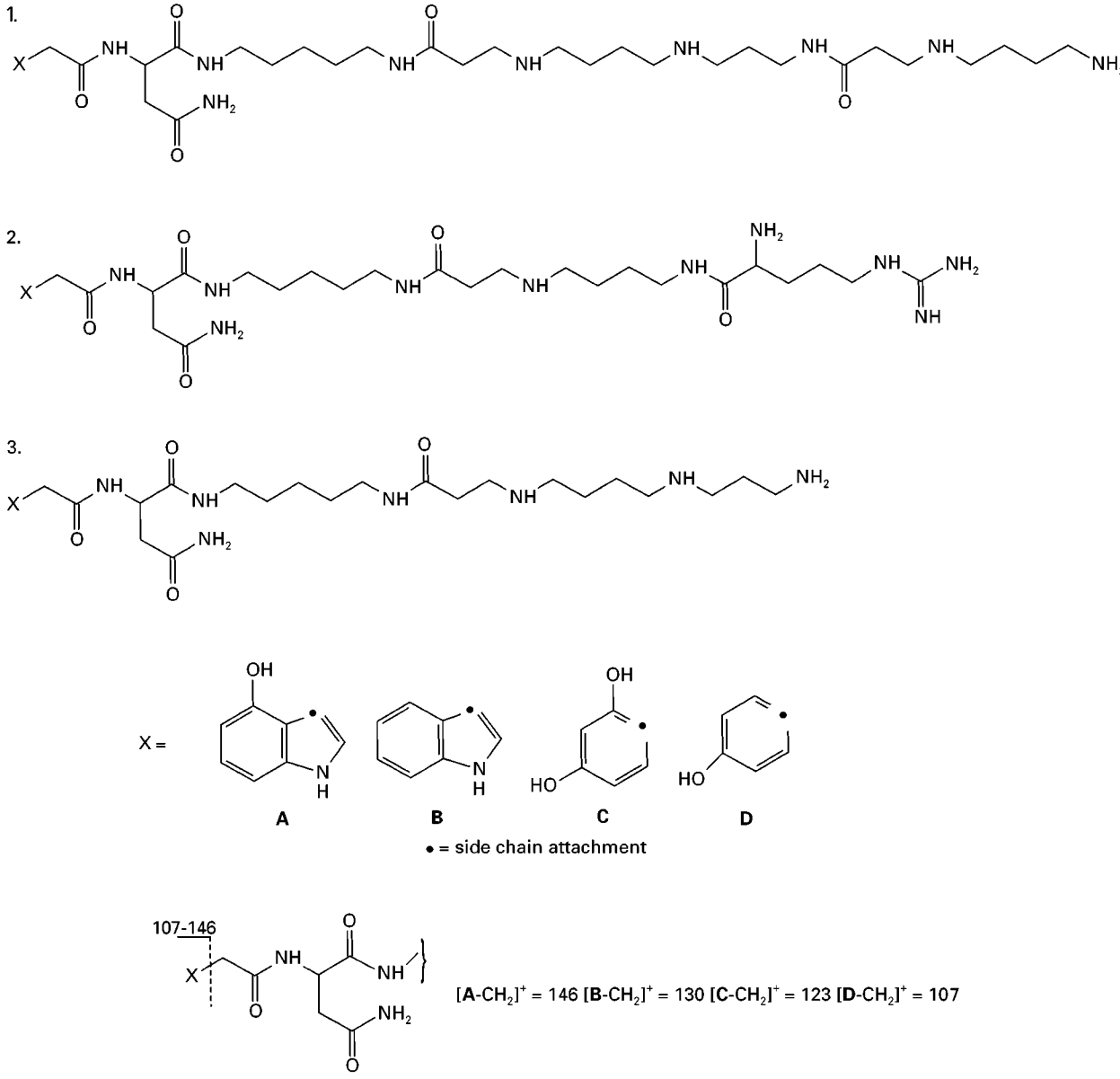

Figure 9 Structures of acylpolyamine toxins from spiders.

mast cells. Toxin identification requires NMR and/or hydrolysis of toxins to amino acids and amines, but for structural confirmation of very small quantities of toxins synthesis has also been employed.

In recent years, major advances have been made possible by the use of μ-column LC with FAB-MS. **Figure 10** shows the two-dimensional MS chromatogram from a spider venom extract obtained using on-line μ-column LC-FAB-MS. The acylpolyamines in this venom have an arginine terminal group with structures similar to those in Figure 9A–2A, –2B and –2C. Further structural information can be obtained using collision-induced dissociation (CID) tandem mass spectrometry (MS-MS) which is often sufficient for the full structural elucidation of these toxins. The aromatic ring in each toxin is readily identified from the strong MS signal because fragmentation at the carbon adjacent to the ring produces ions of m/z 107 (phenol), 123 (dihydroxybenzene), 130 (indole) or 146 (hydroxyindole), as illustrated in Figure 9B. From such studies, over 40 acylpolyamines have been identified in a crude spider venom extract. A combination of LC-MS and matrix-assisted laser desorption/ionization (MALDI) MS has emerged as

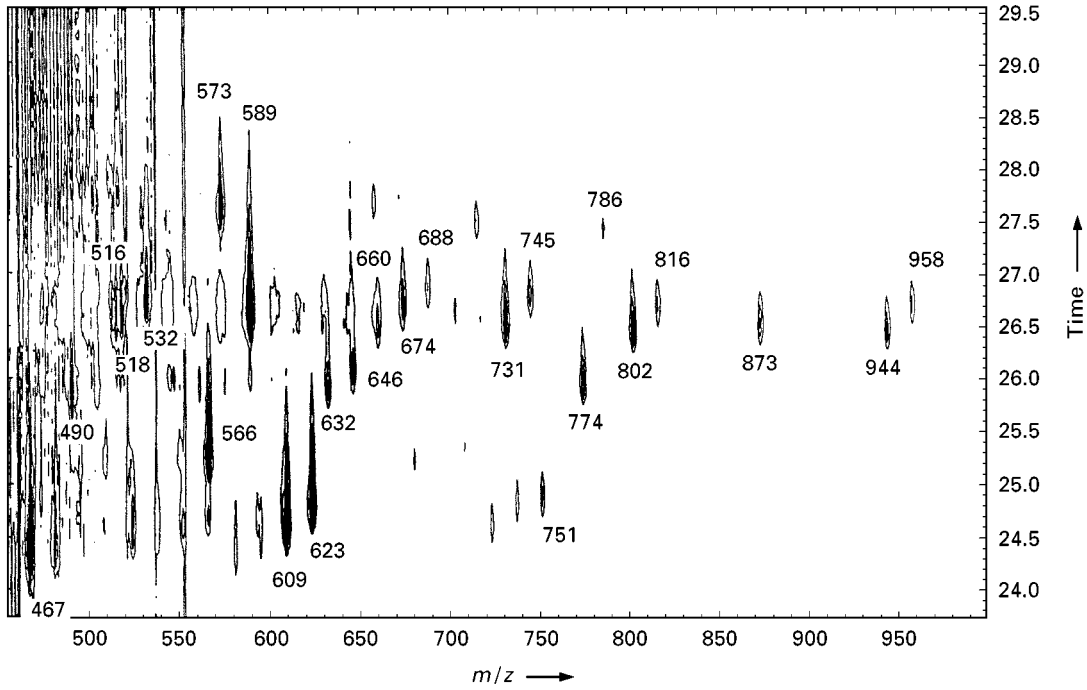

Figure 10 Two-dimensional MS chromatogram display of protonated molecular ions $[M + H]^+$ obtained from spider venom (*Nephilengys cruenta*) extracts using on-line μ-column LC-FAB–MS. (Reproduced with permission from Palma MS *et al.* (1997) *Natural Toxins* 5: 47.)

a powerful technique for the analysis of small samples of venom containing complex mixtures of these toxins.

See Colour Plate 107.

See also: **II/Chromatography: Gas:** Derivatization; Detectors: Mass Spectrometry. **Chromatography: Liquid:** Detectors: Fluorescence Detection; Detectors: Mass Spectrometry. **Electrophoresis:** Capillary Electrophoresis-Mass Spectrometry; One-dimensional Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis. **III/Toxins: Chromatography. Venoms: Chromatography.**

Further Reading

Botana LM, Rodriguez-Vieytes M, Alfonso A and Louzao MC (1996) Phycotoxins: paralytic shellfish poisoning and diarrhetic shellfish poisoning. In: Nollet LML (ed.) *Handbook of Food Analysis*, vol. 2, pp 1147–1169. New York: Marcel Dekker.

Chorus I and Bartram J, eds (1999) *Toxic Cyanobacteria in Water*. World Health Organisation. London: E&FN Spon.

Codd GA, Jefferies TM, Keevil CW and Potter E, eds (1994) *Detection Methods for Cyanobacterial Toxins*. Cambridge: Royal Society of Chemistry.

Falconer I, ed. (1993) *Algal Toxins in Seafood and Drinking Water*. London: Academic Press.

Hu AT, ed. (1988) *Handbook of Natural Toxins*, vol. 3 *Marine Toxins and Venoms*. New York: Marcel Dekker.

James KJ, Furey A, Sherlock IR, *et al.* (1998) Sensitive determination of anatoxin-a, homoanatoxin-a and their degradation products by liquid chromatography with fluorimetric detection. *Journal of Chromatography* 789: 147–157.

McCormick KD and Meinwald J (1993) Neurotoxic acylpolyamines from spider venoms. *Journal of Chemical Ecology* 19: 2411–2413.

Oshima Y (1995) Postcolumn derivatisation liquid chromatographic method for paralytic shellfish toxins. *J AOAC Int.* 78: 528–532.

Palma MS, Itagaki, Y, Fujita T, Naoki H and Nakajima T (1997) Mass spectrometric structure determination of spider toxins: arginine-containing acylpolyamines from venoms of Brazilian garden spider. *Nephilengys cruentata*. *Natural Toxins* 5: 47–57.

Perkins JR, Parker CE and Tomer KB (1993) The characterisation of snake venoms using capillary electrophoresis in conjunction with electrospray mass spectrometry: Black Mambas. *Electrophoresis* 14: 458.

Reguera B, Blanco J, Fernández, ML and Wyatt T, eds (1998) *Harmful Algae*. Santiago de Compestela: Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO.

Satake M, Ofuji K, Naoki H, *et al.* (1998) Azaspiracid, a new marine toxin having unique spiro ring assemblies, isolated from Irish mussels, *Mytilus edulis*. *Journal of the American Chemical Society* 120: 9967–9968.

Schäfer A, Benz H, Fieler W, *et al.* (1994) Polyamine toxins from spiders and wasps. In: Cordell GA and Brossi A (eds) *The Alkaloids: Chemistry and Pharmacology*, vol. 45, pp 1–125. San Diego: Academic Press.