CHAPTER 2.

Chemical and physical characteristics of the principal mycotoxins

Summary

This chapter provides information about the chemical and physical properties of the mycotoxins considered in this book: aflatoxins; fumonisins; ochratoxin A; trichothecenes, especially deoxynivalenol and nivalenol; zearalenone; and ergot alkaloids. This information about structures reveals the chemical diversity of mycotoxins, which is relevant to the wide range of toxicological effects in animals and humans discussed later in the book.

1. Aflatoxins

1.1 Formulae and structures

Aflatoxin B_1. Chemical Abstracts (CA) name: (6aR,9aS)-2,3,6a,9a-tetrahydro-4-methoxycyclopenta[*c*]furo-(3′,2′:4,5]-furo[2,3-*h*][*l*]benzopyran-1,11-dione. Chemical Abstracts Service (CAS) registry number: 1162-65-8. Molecular formula: $C_{17}H_{12}O_6$. Molecular weight: 312.3.

Aflatoxin B_2. CA name: (6aR,9aS)-2,3,6a,8,9,9a-hexahydro-4-methoxycyclopenta[*c*]furo(3′,2′:4,5]furo[2,3-*h*]-[*l*]benzopyran-1,11-dione. CAS registry number: 7220-81-7. Molecular formula: $C_{17}H_{14}O_6$. Molecular weight: 314.3.

Aflatoxin G_1. CA name: (7aR,10aS)-3,4,7a,10a-tetrahydro-5-methoxy-1*H*,12*H*-furo-(3′,2′:4,5]furo[2,3-*h*]pyrano-[3,4-*c*][*l*]benzopyran-1,12-dione. CAS registry number: 1165-39-5. Molecular formula: $C_{17}H_{12}O_7$. Molecular weight: 328.3.

Aflatoxin G_2. CA name: (7aR,10aS)-3,4,7a,9,10,10a-hexahydro-5-methoxy-1*H*,12*H*-furo-(3′,2′:4,5]furo[2,3-*h*]pyrano-[3,4-*c*][*l*]benzopyran-1,12-dione.CAS registry number: 7241-98-7. Molecular formula: $C_{17}H_{14}O_7$. Molecular weight: 330.3.

Aflatoxin M_1. CA name: (6aR,9aR)-2,3,6a,9a-tetrahydro-9a-hydroxy-4-methoxycyclopenta[*c*]furo-(3′,2′:4,5]-furo[2,3-*h*][*l*]benzopyran-1,11-dione. CAS registry number: 6795-23-9. Molecular formula: $C_{17}H_{12}O_7$. Molecular weight: 328.3.

Structures of aflatoxins are shown in Fig. 2.1.

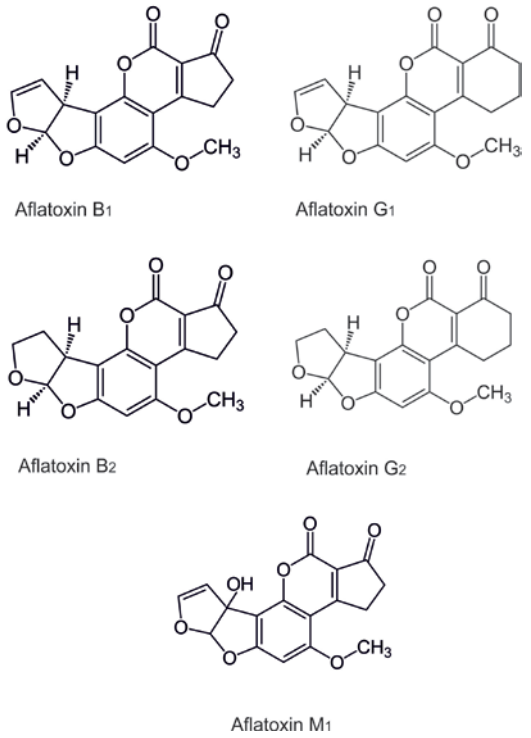
1.2 Physical data

Descriptions. Colourless to pale-yellow crystals. Fluorescence in ultraviolet (UV) light: aflatoxins B_1 and B_2, blue; aflatoxins G_1 and G_2, green; aflatoxin M_1, blue–violet.

Melting-points. See Table 2.1.

Spectral properties. For UV absorption, see Table 2.1. Fluorescence excitation and emission data are not listed in Table 2.1 because they depend on the type of instrument, the solvent, and the supporting media

Fig. 2.1. Structures of aflatoxins



used. For those data, see Wogan (1966), Robertson and Pons (1968), Kiermeier and Kroczek (1974), and Uwaifo *et al.* (1977).

For mass and nuclear magnetic resonance (NMR) spectral data, see Bycroft *et al.* (1970), Stubblefield *et al.* (1970), and Cole and Schweikert (2003).

Specific rotation. $[\alpha]_D$ in chloroform, –558° (aflatoxin B_1), –430° (aflatoxin B_2), –556° (aflatoxin G_1), –473° (aflatoxin G_2); $[\alpha]_D$ in dimethylformamide, –280° (aflatoxin M_1) (Cole and Schweikert, 2003).

1.3 Chemical data

Solubility. Insoluble in non-polar solvents. Slightly soluble in water (10–20 µg/mL). Freely soluble in moderately polar organic solvents (e.g. chloroform, methanol), especially in dimethyl sulfoxide (Cole and Cox, 1981; O'Neil *et al.*, 2001).

Stability. Unstable to UV light in the presence of oxygen. Unstable to extremes of pH (< 3 or > 10). Unstable in the presence of oxidizing agents (Castegnaro *et al.*, 1980, 1991).

Reactivity. Under alkaline conditions, the lactone ring opens and the aflatoxins are apparently absent. However, the reaction is reversible upon acidification.

Ammoniation at high temperature and high pressure opens the lactone ring and results in decarboxylation. This reaction is not reversible.

2. Fumonisins

2.1 Formulae and structures

Fumonisin B_{1}. CA name: 1,2,3-propanetricarboxylic acid, 1,1′-[1-(12-amino-4,9,11-trihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl] ester. CAS registry number: 116355-83-0. Molecular formula: $C_{34}H_{59}NO_{15}$. Molecular weight: 721.

Fumonisin B_{2}. CA name: 1,2,3-propanetricarboxylic acid, 1,1′-[1-(12-amino-9,11-dihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl] ester. CAS registry number: 116355-84-1. Molecular formula: $C_{34}H_{59}NO_{14}$. Molecular weight: 705.

Structures of fumonisins are shown in Fig. 2.2.

2.2 Physical data

Unless otherwise noted, data are from WHO (2000).

Description. White hygroscopic powder.

Melting-point. Not known (compounds have not been crystallized).

Spectral properties. For mass and NMR spectral data, see Bezuidenhout *et al.* (1988), Laurent *et al.* (1989), Plattner *et al.* (1990), Savard and Blackwell (1994), and Cole *et al.* (2003a).

2.3 Chemical data

Solubility. Soluble in methanol, in acetonitrile–water, and in water (at least 20 g/L) (NTP, 2001).

Stability. Stable in acetonitrile–water (1:1) at 25 °C. Unstable in methanol at 25 °C, forming monomethyl and dimethyl esters (Gelderblom *et al.*, 1992; Visconti *et al.*, 1994). Stable in methanol at –18 °C (Visconti *et al.*, 1994). Stable in buffer solutions over the pH range 4.8–9 at 78 °C (Howard *et al.*, 1998).

Octanol–water partition coefficient for fumonisin B_{1}. log P = 1.84 (Norred *et al.*, 1997).

3. Ochratoxin A

3.1 Formula and structure

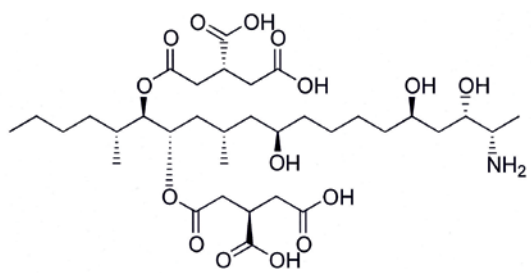
Ochratoxin A. CA name: *N*-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-7-yl)carbonyl]-L-phenylalanine. CAS registry number:

Table 2.1. Melting-points and ultraviolet absorption of aflatoxins

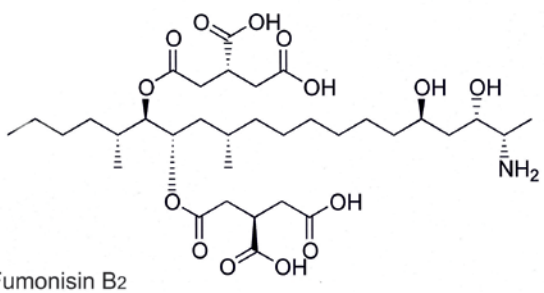
Aflatoxin	Melting-point (°C)	Ultraviolet absorption	
		λ_{max}(nm)	ε (L·mol^{-1}·cm^{-1}) × 10^{-3}
B_1	268–269 (decomposition) (crystals from $CHCl_3$)	223	25.6
		265	13.4
		362	21.8
B_2	286–289 (decomposition) (crystals from $CHCl_3$–pentane)	265	11.7
		363	23.4
G_1	244–246 (decomposition) (crystals from $CHCl_3$–methanol)	243	11.5
		257	9.9
		264	10.0
		362	16.1
G_2	237–240 (decomposition) (crystals from ethyl acetate)	265	9.7
		363	21.0
M_1	299 (decomposition) (crystals from methanol)	226	23.1
		265	11.6
		357	19.0

Data from O'Neil *et al.* (2001).

Fig. 2.2. Structures of fumonisins



Fumonisin B_1



Fumonisin B_2

303-47-9. Molecular formula: $C_{20}H_{18}ClNO_6$. Molecular weight: 403.8.

The structure of ochratoxin A (OTA) is shown in Fig. 2.3.

3.2 Physical data

Description. White odourless crystalline solid (Pohland *et al.*, 1982). Intensely fluorescent in UV light, emitting green and blue fluorescence in acid and alkaline solutions, respectively, due to two different forms, i.e. closed or open lactone ring, respectively.

Melting-point. 159 °C when recrystallized from benzene–hexane (Natori *et al.*, 1970); 169 °C when recrystallized from xylene (Van der Merwe *et al.*, 1965a, 1965b); 168–173 °C after drying for 1 hour at 60 °C (Pohland *et al.*, 1982).

Specific rotation. $[\alpha]^{20}_D$ –118° (c = 1.1 mmol/L in chloroform) (Van der Merwe *et al.*, 1965a, 1965b); $[\alpha]^{21}_D$ –46.8° (c = 2.65 mmol/L in chloroform) (Pohland *et al.*, 1982).

UV spectrum. At λ_{max} of 214, 282, and 332 nm, extinction coefficients of 37.2×10^{-3}, 0.89×10^{-3}, and 63.3×10^{-3} $L \cdot mol^{-1} \cdot cm^{-1}$, respectively, have been reported (Cole and Cox, 1981).

Other spectral properties. For infrared (IR) spectra, see Van der Merwe *et al.* (1965a, 1965b), Steyn and Holzapfel (1967), and Pohland *et al.* (1982). For NMR spectra, see Pohland *et al.* (1982) and Cole *et al.* (2003b). For mass spectra, see Pohland *et al.* (1982) and Cole *et al.* (2003b).

3.3 Chemical data

Solubility. Moderately soluble in polar organic solvents (e.g. chloroform, ethanol, methanol).

Stability. OTA is partially degraded under normal cooking conditions (Müller, 1983). The stability of OTA to heating conditions depends on the water activity of the medium (Subirade, 1996; Van der Stegen *et al.*, 2001).

Reactivity. The lactone ring opens under alkaline conditions, but the reaction is reversible. Solutions of OTA are completely degraded by treatment with an excess of sodium hypochlorite.

4. Deoxynivalenol

4.1 Formula and structure

Deoxynivalenol. CA name: 12,13-epoxy-3,7,15-trihydroxy-(3α,7α)-trichothec-9-en-8-one. CAS registry number: 51481-10-8. Molecular formula: $C_{15}H_{20}O_6$. Molecular weight: 296.32.

The structure of deoxynivalenol (DON) is shown in Fig. 2.4.

4.2 Physical data

Description. White needles.

Melting-point. 151–153 °C.

Specific rotation. $[\alpha]^{20}_D$ +6.35° (c = 0.07 mmol/L in ethanol).

Spectral properties. IR, UV, NMR, and mass spectral data have been reported (Cole and Cox, 1981; Cole *et al.*, 2003c).

4.3 Chemical data

Solubility. Soluble in chloroform, ethanol, methanol, and ethyl acetate.

Stability. Autoclaving creamed maize reduced DON content by only 12% (Wolf-Hall *et al.*, 1999). At pH 4.0, DON appeared to be very stable, showing no destruction at 100 °C or 120 °C and only partial destruction at 170 °C after 60 minutes. At pH 7.0, DON was still stable but showed more destruction at 170 °C after 15 minutes. At pH 10.0, DON was partially destroyed at 100 °C after 60 minutes and was totally destroyed at 120 °C after 30 minutes and at 170 °C after 15 minutes (Wolf and Bullerman, 1998).

When DON was gamma-irradiated on maize, breakdown of DON began only after irradiation to 20 kGy, and 80–90% of the DON remained after irradiation to 50 kGy (O'Neill *et al.*, 1993).

No significant decomposition of DON was observed when stored in ethyl acetate for 24 months at 25 °C or 3 months at 40 °C (Widestrand and Pettersson, 2001). DON was relatively stable in buffer solutions over the pH range 1–10 (Lauren and Smith, 2001).

5. Nivalenol

5.1 Formula and structure

Nivalenol. CA name: 12,13-epoxy-3,4,7,15-tetrahydroxy-(3α,4β,7α)-trichothec-9-en-8-one. CAS registry number: 23282-20-4. Molecular formula: $C_{15}H_{20}O_7$. Molecular weight: 312.32.

The structure of nivalenol (NIV) is shown in Fig. 2.4.

5.2 Physical data

Description. White crystals.

Melting-point. 222–223 °C (with decomposition, after drying in the presence of P_2O_5 at reduced pressure).

Specific rotation. $[\alpha]^{20}_D$ +21.54° (c = 1.3 mmol/L in ethanol).

Spectral properties. IR, UV, NMR, and mass spectral data have been reported (Cole and Cox, 1981; Brumley *et al.*, 1982; Cole *et al.*, 2003c).

5.3 Chemical data

Solubility. Soluble in chloroform, ethanol, methanol, and ethyl acetate; slightly soluble in water; soluble in polar organic solvents (Budavari, 1989).

Stability. No significant decomposition of NIV was observed when stored in ethyl acetate for 24 months at 25 °C or for 3 months at 40 °C. A significant decrease of NIV stored

Fig. 2.3. Structure of ochratoxin A

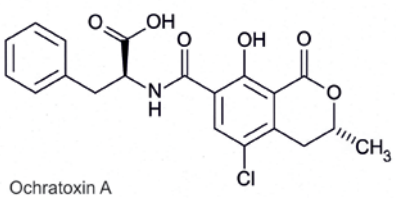


Fig. 2.4. Structures of major trichothecenes

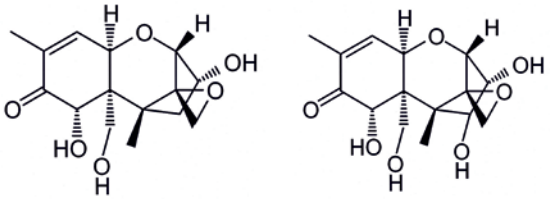


Fig. 2.5. Structure of zearalenone

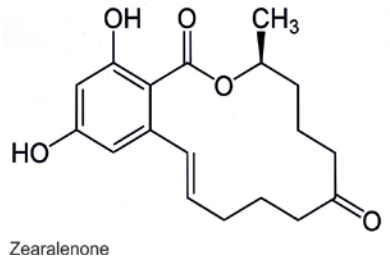
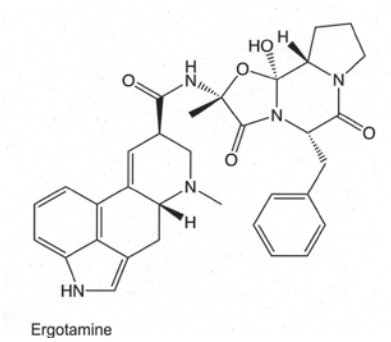


Fig. 2.6. Structure of ergotamine



as a thin film was observed after 9 months at 25 °C (Widestrand and Pettersson, 2001). NIV is relatively stable in buffer solutions over the pH range 1–10 (Lauren and Smith, 2001).

6. Zearalenone

6.1 Formula and structure

Zearalenone. CA name: 3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl-1*H*-2-benzoxacyclotetradecin-1,7(8*H*)-dione. CAS registry number: 17924-92-4. Molecular formula: $C_{18}H_{22}O_5$. Molecular weight: 318.4.

The structure of zearalenone (ZEA) is shown in Fig. 2.5.

6.2 Physical data

Description. White crystals.

Melting-point. 164–165 °C.

Specific rotation. $[\alpha]^{25}_D$ –170.5° (c = 1.0 mmol/L in methanol); $[\alpha]^{21}_D$ –189° (c = 3.14 mmol/L in chloroform).

Spectral properties. IR, UV, proton NMR, and mass spectral data have been reported (Cole and Cox, 1981). The molar absorptivities of ZEA in acetonitrile at 236, 274, and 314 nm were established, and a common reference wavelength of 274 nm with molar absorptivity of 12 623 ± 111 $L \cdot mol^{-1} \cdot cm^{-1}$ was recommended for ZEA in acetonitrile (Josephs *et al.*, 2003).

6.3 Chemical data

Solubility. Solubilities at 25 °C in percent by weight are: water, 0.002; *n*-hexane, 0.05; benzene, 1.13; acetonitrile, 8.6; dichloromethane, 17.5; methanol, 18; ethanol, 24; and acetone, 58 (Hidy *et al.*, 1977).

Stability. ZEA was stable when heated at 120 °C; 29% decomposed when heated at 150 °C for 60 minutes and 69% when heated at 200 °C for 60 minutes (Kuiper-Goodman *et al.*, 1987). Stable to hydrolysis in neutral or acid buffer solutions (Müller, 1983). Less than 23% of ZEA was lost when heated in aqueous buffer solution to 125 °C for 60 minutes, but 34–68% was lost after 60 minutes at 150 °C, depending on the pH of the buffer. More than 92% was lost after 60 minutes at 175 °C, and complete loss was observed in < 30 minutes at 225 °C, regardless of pH. ZEA was most stable at pH 7, and the greatest losses occurred above 175 °C (Ryu *et al.*, 2003).

Extrusion cooking of maize grits resulted in significant reductions of ZEA with either mixing screws or non-mixing screws, but use of mixing screws was somewhat more effective (66–83% reduction) overall than non-mixing screws (65–77%). Greater reduction of ZEA content was observed at either 120 °C or 140 °C than at 160 °C (Ryu *et al.*, 1999).

ZEA content was not reduced by heating at 110 °C for 12 days after treatment with a sodium bicarbonate solution (Lauren and Smith, 2001).

7. Ergot alkaloids

Ergots, the sclerotia produced by *Claviceps purpurea* and related species, contain a remarkable variety of compounds, which can be divided into three groups: derivatives of lysergic acid, derivatives of isolysergic acid, and clavines. The most important of these is ergotamine.

7.1 Formula and structure

Ergotamine. CA name: 12′-hydroxy-2′-methyl-5′-(phenylmethyl)-ergotaman-3′,6′,18-trione. CAS registry number: 113-15-5. Molecular formula: $C_{33}H_{35}N_5O_5$. Molecular weight: 581.66.

The structure of ergotamine is shown in Fig. 2.6.

7.2 Physical data

Description. White powder.

Melting-point. 180 °C.

Spectral properties. UV, IR, and fluorescence spectral data were reviewed by Hofmann (1964). The electron mass spectrum of ergotamine was described by Vokoun and Řehāček (1975), and the [^{1}H]-NMR spectrum was reported by Pierri *et al*. (1982).

Specific rotation. $[\alpha]^{20}_D$ –160°.

7.3 Chemical data

Solubility. Some data on recrystallization, appearance, and solubility were reviewed by Hofmann (1964).

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