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The coming of age of azadirachtins and related tetranortriterpenoids

Hans E. Hummel[1,2], D.F. Hein[1] and H. Schmutterer[3]

ABSTRACT

Azadirachtins (azas) are known as a family of natural phagorepellents and antifeedants isolated from the seeds of the neem tree *Azadirachta indica* A. Juss. (Meliaceae). They exert a strong negative influence on behavior (feeding and mating activity), postembryonic development (molts), and fecundity of insects resulting in significant reduction of general fitness. The history of discovery and characterization of azas as a unique family of natural products was full of surprises and obstacles. But after the labors lasting 4 decades in various labs in Europe (Kraus, Schmutterer, Butterworth and Morgan, Ley) and in North America (Nakanishi), the complicated multifunctional tetranortriterpenoid aza A emerged and was characterized by elemental composition of $C_{35}H_{44}O_{16}$. The structure proposals, although arrived at independently in competition and by completely different methods (NMR and X-ray analysis), ultimately converged into a commonly accepted structure individually published side by side in 1987. It was reconfirmed by Veitch *et al*. in 2007 in Ley's group in Cambridge, England, through total synthesis and therefore can be considered as ultimate proof and as valid without any remaining doubt. The 25th anniversary of this scientific achievement is reason for celebration. A new member of the azadirachtin family was discovered at Giessen in 1991 and named marrangin. It occurs in seeds of the marrango tree *Azadirachta excelsa* (Jack) Jacobs and has the elemental composition of $C_{35}H_{44}O_{15}$. In some insect species but also in mites like *Tetranychus urticae*, its biological activity is significantly superior to azadirachtin A. The value of these biorational compounds, with their very low vertebrate toxicity and their low toxicity to insect members of the third level of the food chain, today is globally recognized. Quite recently, azas and analogs are gaining increasing acceptance in veterinary and human medicine.

Key words: *Azadirachta indica* A. Juss., *A. excelsa,* azadirachtin, biopesticides, biorationals, *Epilachna varivestis,* marrangin, marrango, neem tree, organic agriculture, *Tetranychus urticae*

INTRODUCTION

Significance of the azadirachtin family of natural products

Azadirachtin (aza) is a natural antifeedant, insect growth regulator, and sterilant found in the seeds of the neem tree, *Azadirachta indica* (Meliaceae). The correct structure was for the first time determined by NMR (Kraus *et al*., 1985) and confirmed by X-ray analysis of a derivative, detigloyldihydoazadirachtin (Broughton *et al*., 1986). In 1987 three research groups (Bilton *et al*., 1987; Kraus *et al*., 1987; Turner *et al*.,1987) simultaneously published the complete history of the structure determination process of azadirachtin and some related compounds). Recently, the group of Ley at the University of Cambridge, England, decisively confirmed the structure by the total synthesis of the rather complicated molecular architecture of this tetranortriterpenoid compound (Veitch *et al*., 2007) (Figure 1A). Aza was first identified by bioassay and isolated by Butterworth and Morgan (1968; 1972); Kalinowski *et al*. (1993) isolated and determined the structure of a close analogue of azadirachtin, called marrangin (azadirachtin L) (Figure 1B), from *A. excelsa* seeds. A number of biosynthetically related compounds (Rembold, 2002) in the seeds of both trees are also active and are sometimes known by the general term azas. The value of these biorationals (Ishaaya and Horowitz, 2009) for insect and mite pest management without any appreciable vertebrate toxicity was recognized early on (Schmutterer, 1988,1990,2002,2005; Ascher *et al*., 2002). In spite of their comparatively high price, azas are strongly favoured by the organic farming community (Hummel *et al*., 2008) where only very few compounds meet the highly restrictive standard for field applications. Recently, azas have been suggested for use in veterinary science and medicine and are now beginning to be a commercial success (Schmutterer, 2005; Kleeberg and Strang, 2009). In spite of the comparably high price of commercial products numerous applications in organic agriculture are known where only very few compounds are compatible at all with restrictive consumer standards and moreover have been registered for use

(Hummel *et al*., 2008). In addition, azadirachtin is of basic importance for studies in hormone physiology and neuroendocrinology (Mordue (Luntz), 2002; Rembold, 2002; 2004).

In addition to field applications, azas are also important probes for mechanistic studies in basic biology, insect physiology and neuro-endocrinology (Mordue and Blackwell, 1993; Mordue (Luntz) 2002; Rembold, 2002,2004; Kraus, 2002). Applied as a mixture of natural products from the seeds, azas are a valuable alternative to synthetic compounds with their ever present propensity for developing resistance in case of widespread and indiscriminate use. Azas with their multiple mechanisms of action are virtually insensitive to developing resistance even under conditions of strong selection pressure.

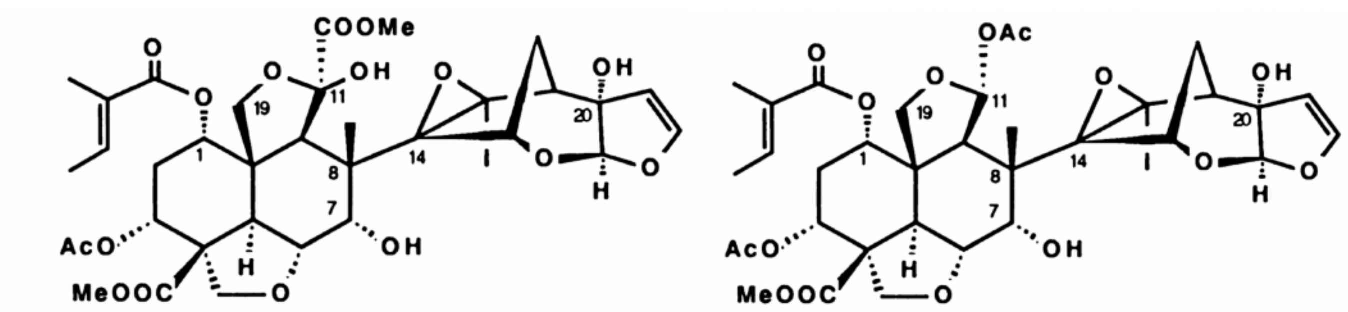
RESULTS

General review

The neem and marrango trees (*A. indica* and *A. excelsa*) are rich sources of tetranortriterpenoid natural products, with compounds of value for insect, virus, mite, nematode, fungal, and bacterial management (Schmutterer, 2002). Butterworth and Morgan (1968,1972) studied feeding inhibition of extracts of neem seeds in desert locusts and aided by this sensitive bioassay, succeeded in isolating aza. The first correct structural assignment of a crystalline derivative closely related to azadirachtin was published just 25 years ago (Broughton *et al*., 1986) after 18 years of efforts to determine all structural details. X-ray crystallography was the method of choice. The structure of azadirachtin itself (Figure 1A) was established chiefly but independently by NMR methods. Structural results were published by the groups of Kraus in Germany (Kraus *et al.*, 1985,1987), Ley in England (Broughton *et al*., 1986; Bilton *et al.*, 1987), and Nakanishi in the USA (Turner *et al*., 1987). Six years later, the structure of marrangin was established by Kalinowski *et al*. (1993) (Figure 1 B) who again used both ^{1}H and ^{13}C-NMR methods. Mordue and Blackwell (1993) and Mordue (Luntz) (2002) reviewed the knowledge available on the mode of action. Schmutterer (1988,1990) published exhaustively on comparative efficacies of azas in various insect species.

The close similarity of the structures in Figure 1A and 1B is evident. Differences exist only in the functional groups attached to carbon 11. It seems that azas (of which a series of at least two dozen has been identified) (Goviadachari *et al*., 1992a,b; Kraus 2002; Siddiqui *et al*., 2006) have more than one mechanism of action. In fact, they are characterized by multiple activities at the larval, pupal and adult level, show differences in different insects (Schmutterer, 1988, 1990) and arthropods (Table 1) and affect both behavioural and developmental reactions. Azas interfere with RNA synthesis and insect brain hormone metabolism (Rembold, 2004). Thus, they indirectly modify both the synthesis of and the responses to steroid and juvenile hormones, and to pheromones (Hummel, 1989a; b).

Also, the potential applications in human and veterinary medicine are numerous (Schmutterer, 2002; Talwar *et al*., 2002; Kleeberg and Strang, 2009; van der Esch *et al*., 2009; Ketkar, 2009). Effects of neem on many pest insect species have been investigated by Schmutterer (1988,1990); effects on the hormone physiology of insects have been described (Mordue and Blackwell, 1993; Mordue, 2002; Rembold, 2004), together with studies of their neuroendocrinology. In addition to the Indian neem tree, the genus *Azadirachta* contains the related Thai neem, *A. siamensis* (Sombatsiri *et al*., 2002) which can hybridize with *A. indica*. The botanically also related *A. excelsa* (marrango) (Hein, 1999; Schmutterer, 2002) for some insects shows effects more pronounced than those of Indian neem itself. Semi-preparative purification of the natural



(A) $C_{35}H_{44}O_{16}$ (B) $C_{35}H_{44}O_{15}$

Figure 1. Molecular structure and elemental composition of (A) azadirachtin and (B) marrangin, partly taken and modified from the report of the National Research Council (1992).

Table 1. EC_{50} and growth inhibition data in insects and mites of azadirachtin A and marrangin from various authors.

Compound		Test organisms	Biological activity (µg/g)	References
Azadirachtin A	Insects	*Epilachna varivestis*	A: EC50 = 25,7 µg/ml	Hein, 1999
			L: EC50 = 1,5 µg/ml	Hein, 1999
		Spodoptera littoralis	A: EC50 = 1,6 µg/ml	Hein, 1999
			G: 20-30% at 10 ppm	Kraus, 2002
	Mites	*Tetranychus urticae*	L: 50% at 80 ppm	Martinez-Villar *et al.*, 2005
			R0: 0.79 at 80 ppm (control: 14.40)	
		Phytoseiulus persimilis	M: 40% female toxicity (4.5 g a.i./hl)	Duso *et al.*, 2008
Marrangin (Azadirachtin L)	Insects	*Epilachna varivestis*	G: EC50 = 0,25 ppm	Ermel, 1991
			L: 100% at 1 ppm	Ermel, 1991
	Mites	*Tetranychus urticae*	M: 1.2% (protonymphes)	Sanguanpong, 1992
			S: 0.2% (protonymphes)	Doll and Schmutterer, 1993
		Phytoseiulus persimilis	0.45 ppm	Sanguanpong, 1992

A = antifeedant activity; L =larvicidal activity; EC = effective concentration; F = fecundity; G = growth inhibition; M = mortality; N= number of eggs deposited per female; S = survival rate;

products of *A. indica* and *A. excelsa* has been accomplished by column chromatography, but also by multilayer countercurrent chromatography (MLCCC) (Hummel *et al.*, 1997). Within the last twenty five years, methods for the analysis of azadirachtin and other azas including their quantification by various chromatographic, spectroscopic, immunological (Schütz *et al.*, 1997), and bioassay methods (Hein, 1999) have been published. Applications of neem ingredients in organic agriculture and integrated pest management have been described by Hummel (1989a,1989b, 2006) and Hummel *et al.* (2007, 2008) and also by Schmutterer (2005). Selected data of the biological activity of azadirachtin A and azadirachtin L (marrangin) against agricultural pests are listed in table 1.

DISCUSSION

Azadirachtin and other azas are highly oxidised tetranortriterpenoid natural products with many functional groups and numerous asymmetric centers. Only due to recent advances of modern analytics and toxicology do we have the tools to successfully work with them today (Allan *et al.*, 1994; Wewetzer, 1999; Schmutterer, 2002; Morgan, 2006). The truely international effort of neem research should be emphasized. Many different laboratories of many countries contributed to the immense knowledge on neem available today. After the classical comprehensive book account of Schmutterer (2002), there have been a number of contributions with review character by Isman (2006), by the Chinese editors of the Neem symposium volume published at Kunming (2006), and Kleeberg and Strang (2009). On the 25th anniversary of their unequivocal structural identification it is time to remember the accomplishments that have been achieved and to look ahead to the possibilities that still may be ahead of us.

CONCLUSIONS AND OUTLOOK

Knowledge of azadirachtin and its analogues contributed immensely to basic entomological research, insect endocrinology and applied aspects of pest management. The primary literature on azadirachtin and neem lists 10,740 titles (CAB abstracts, status March 13, 2011). Azadirachtins represent a major group of biorationals whose impact on plant- and stored product protection (Saxena, 2002) as well as in veterinary (Schmutterer and Huber, 2005; Ketkar, 2009; Kleeberg and Strang, 2009; van der Esch *et al*., 2009), and human medicine (Schmutterer, 2002) including pharmacology (Ketkar and Ketkar, 2002) is beginning to emerge and to be appreciated worldwide. Western scientific efforts can learn a lot from traditional knowledge of people in Asia who for millennia cherished the neem tree as a gift of the gods (Ahmed and Grainge, 1986; Ahmed, 2002; Koul, 2004).

The first twenty five years of azadirachtin were a period of breathtaking discoveries and scientific accomplishments. The next twenty five years to come should be expected to see major advances in sustainable applications for the welfare of humans, animals, for global agriculture, and – significantly – toward the improvement of the fortune of the underprivileged (Hellpap *et al*., 2002).

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Hans E. Hummel[12*], D.F. Hein[1] and H. Schmutterer[3]

1,2-Chair of Organic Agriculture, Justus-Liebig-University Giessen, Germany.

3-Institute of Chemistry, University of Hohenheim, Stuttgart-Hohenheim, Germany

*E-mail: Hans.E:Hummel@agrar.uni-giessen.de

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