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Twenty five years of azadirachtins (1986-2011)

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Abstract: Azadirachtins are known as phagorepellent natural products from seeds of the neem tree *Azadirachta indica* A. Juss (Meliaceae), which impede the development of larval insects and sterilize adults. The labors of the past three decades ultimatively lead to a chemical structure, which has been unanimously but independently elaborated by German, British and American authors. This structure is in force ever since 1985/87 and was ultimately confirmed by Veitch & al. in Ley's group in Cambridge, England, through total synthesis. Its 25th birthday is presently being celebrated.
Marrangin (=Azadirachtin L) was discovered in Gießen and chemically identified by Kalinowski and colleagues. It occurs in seeds of the marrango tree, *Azadirachta excelsa* (Jack) Jacobs. 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 today is globally recognized. Quite recently, azadirachtin and analogues gain acceptance in veterinary and human medicine.

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

INTRODUCTION

In 1987 three research groups simultaneously published the complete molecular structure of azadirachtin, a natural antifeedant, insect growth regulator, and sterilant found in the seeds of the neem tree, *Azadirachta indica* (Meliaceae). Recently, the group of S. Ley at the University of Cambridge, England, decisively confirmed the structure by the total synthesis of the rather complicated molecular architecture of the tetranortriterpenoid compound [1] (Figure 1A). It was first characterized and isolated by Butterworth and Morgan [2, 3]. Kalinowski and colleagues isolated and determined the structure of a close analogue of azadirachtin, called marrangin (azadirachtin L) (Figure 1B), discovered in Gießen [4], from *A. excelsa* seeds [5]. A number of biosynthetically related compounds in the seeds of both trees are also active and are sometimes known by the general term azas. The value of these biorationals for insect pest management without any appreciable vertebrate toxicity was recognized early by Prof. Schmutterer [6-12], and their low toxicity to members of the third level of the food chain today is, today is globally recognized. In spite of their comparatively high price, azas are strongly favoured by the organic farming community [13] 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 [12, 14]. In spite of the comparably high price of the commercial products, applications in organic agriculture are known, are compatible with the very few registered commercial insecticides, and have been used in various agricultural systems [13]. In addition, azadirachtin is of basic importance for studies in human physiology and neuroendocrinology [15, 16].

In addition to field applications, azas are also important probes for mechanistic studies in basic biology, insect physiology and neuro-endocrinology [15, 17-19]. Applied as a mixture of natural products from the seeds, azas are a valuable alternative to synthetic compounds with their propensity for developing resistance. Azas with their multiple mechanisms of action are virtually insensitive to developing resistance even under condition of strong selection pressure.

MATERIALS and METHODS

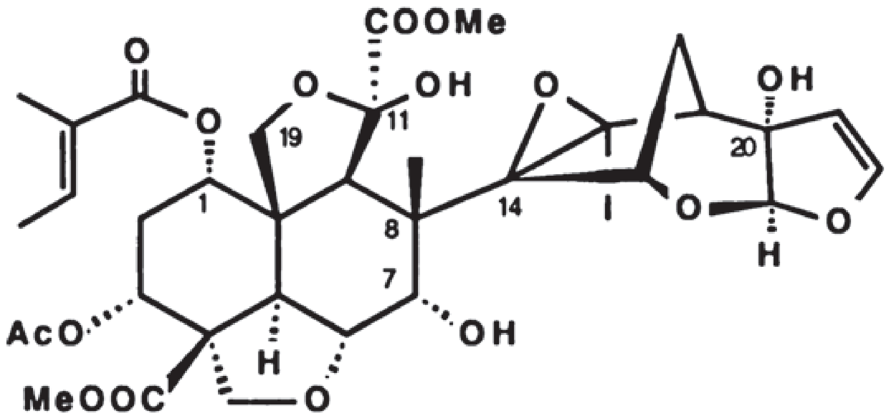
Guidelines for finding specific experimental methods are listed in various chapters of Schmutterer [8] and in multiple references cited herein.

General review of results

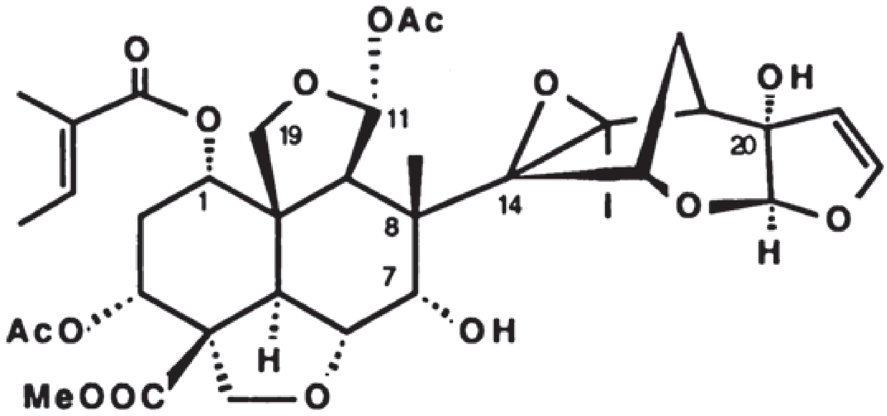
The neem and marrango trees (*A. indica* and *A. excelsa*) are rich sources of tetranortriterpenoid natural products, which are of value for insect, virus, mite, nematode, fungal, and bacterial management [8]. Butterworth and Morgan studied the feeding inhibition of neem seed extracts in desert locusts and by the aid of this sensitive bioassay they succeeded in isolating aza [3]. The first structural assignment of a crystalline derivative closely related to azadirachtin was published 25 years ago [20] 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 by the use of NMR methods. Structural results were published simultaneously by the groups of Ley in England [21], Kraus in Germany [22] and Nakanishi in the USA [23]. Six years later, the structure of marrangin was established by Kalinowski and colleagues [5] (see Figure 1 B) who again used NMR methods. Mordue & Blackwell [17] and Mordue (Luntz) [15] reviewed the knowledge available on the mode of action. Schmutterer [10, 11] published the comparative toxicity 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 have more than one mechanism of action. In fact, they are characterized by multiple activities at the larval, pupal, and adult stages and affect both behavioural and developmental processes. Azas interfere with RNA synthesis and insect brain hormone metabolism [16] and thus indirectly modify both the synthesis and the response to pheromones [24, 25].

In addition, the potential applications in human and veterinary medicine are numerous [8, 14, 27-29]. Effects of neem on many pest insect species have been investigated by Schmutterer [10, 11] along with its effect on the hormonal system of insects [15-17]. In addition to the Indian neem tree, the genus *Azadirachta* contains a closely related Thai neem, *A. indica* var. *siamensis* [30], while the botanically related *A. excelsa* (marrango) [8, 31]shows more pronounced effect than the Indian neem. Semi-preparative purification of the natural products of *A. indica* and *A. excelsa* has been accomplished by column chromatography and by multilayer countercurrent chromatography (MLCCC) [32]. Within the last twenty five years, methods for the analysis of azadirachtin and other azas including their quantification, by various chromatographic, spectroscopic, immunological [33], and bioassay methods [31] have been published. Applications of neem components in organic agriculture and integrated pest management have been described by [12, 13, 24-26, 34, 35].



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



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

Figure 1. Molecular structure and atomic composition of (A) azadirachtin and (B) marrangin, partly taken and modified from [26].

Selected data of the biological activity of azadirachtin A and azadirachtin L (marrangin) against agricultural pests are listed in Table 1.

Table 1. EC50 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	[32]
			L: EC50 = 1,5 μg/ml	[32]
		Spodoptera littoralis	A: EC50 = 1,6 μg/ml	[32]
			G: 20-30 % at 10 ppm	[48]
	Mites	*Tetranychus urticae*	L: 50 % at 80 ppm R0: 0.79 at 80 ppm (control: 14.40)	[49]
		Phytoseiulus persimilis	M: 40 % female toxicity (4.5 g a.i. / hl)	[50]
Marrangin (Azadirachtin L.)	Insects	*Epilachna varivestis*	G: EC50 = 0,25 ppm	[48]
			L: 100 % at 1 ppm	[48]
	Mites	*Tetranychus urticae*	M: 1.2% (protonymphs) S: 0.2% (protonymphes)	[51] [52]
		Phytoseiulus persimilis	0.45 ppm	[51]

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;

DISCUSSION

Azadirachtin and other azas are highly oxidised tetranortriterpenoid natural products with many functional groups and numerous asymmetric centers. Due to recent advances of modern analytics and toxicology we have today the tools to successfully work with such interesting compounds [36-38]. 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 many applied aspects of pest management in various parts of the world [39-44]. 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 as well as in veterinary [14, 28, 29, 45] and human medicine [8, 27] is beginning to emerge and to be appreciated worldwide. Western scientific efforts can profit from traditional knowledge of people in Asia who for millennia cherished the neem tree as a gift of the gods [7, 46, 47].

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 neem product applications in various fields.

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