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Investigating the Structure–Activity Relationship of the Insecticidal Natural Product Rocaglamide

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Abstract: The natural product Rocaglamide (**1**), isolated from the tree *Aglaia elliptifolia*, is a compelling but also challenging lead structure for crop protection. In laboratory assays, the natural product shows highly interesting insecticidal activity against chewing pests and beetles, but also phytotoxicity on some crop plants. Multi-step syntheses with control of stereochemistry were required to probe the structure–activity relationship (SAR), and seek simplified analogues. After a significant research effort, just two areas of the molecule were identified which allow modification whilst maintaining activity, as will be highlighted in this paper.

Keywords: Insecticide · Rocaglamide · Structure–Activity Relationship

The first report by King *et al*. on the isolation of Rocaglamide (**1**) (Fig. 1) from the tree *Aglaia elliptifolia*, together with observed anti-leukemic activity appeared in 1982.[1] This publication stimulated research in many groups, and during the following twenty years, more than sixty closely related natural products have been isolated and characterized. An excellent summary on the chemistry and biology of these natural products has recently appeared.[2] Many of these natural products have been reported to exhibit interesting insecticidal activity against a number of insect pests, and we have also reported that Rocaglamide shows interesting herbicidal activity.[3] In addition to crop protection, the anti-leukemic activity continued to interest medicinal chemistry groups, with recent discoveries on the mode of action of such compounds offering new perspectives.[4]

With limited amounts of such compounds being available from natural sources, synthetic chemists quickly took up the challenge to prepare Rocaglamide and related compounds. A recently published review nicely summarizes the various approaches developed,[5] which was followed by a further publication.[6] We were keen to prepare synthetic analogues of Rocaglamide to explore the potential for crop protection, and report herein our findings on the insecticidal activity and structure–activity relationship (SAR). We also demonstrate the importance of chirality, through chiral separation and screening of enantiomers.

The insecticidal activity of Rocaglamide and related natural products has been published;[2] insecticidal and anti-feeding activity has been reported against certain lepidopteran larvae such as *Spodoptera littoralis* boisd (African or Egyptian cotton leafworm), *Pieris rapae* (small cabbage white), *Helicoverpa armigera* (African cotton bollworm), *Plutella xylostella* L (Diamondback moth), and *Ostrinia nubilis* (European corn borer). We have reported the insecticidal activity of Rocaglamide (**1**) (Fig. 1) and related natural products, and shown the activity to be highly comparable to today's chemical standards.[7] However, only a few reports concerning the SAR of these natural products have appeared in the literature, and have dealt with structural changes observed around the cyclopentyl ring. Natural products bearing a methoxy substituent at position 8b were reported to be inactive; acylation of the C-1 alcohol reduced activity, whereas modifications to the amide substituent C-2 retained activity. Inverting the stereochemistry at C-2 led to similar levels of activity against *Plutella xylostella*, but greatly reduced activity against other pests.[8]

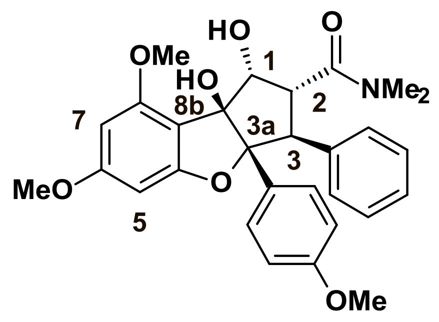

Fig. 1. Structure of Rocaglamide (**1**).

Structure–Activity Relationship Investigations

A number of complimentary synthetic routes have been developed for the total synthesis of Rocaglamide and related compounds. For the purposes of this study, the synthetic approach first reported by Taylor,[9] and later by Dobler *et al*.[10] was used, details of which will not be discussed here.

We first investigated the impact of structural modifications around the cyclopentyl ring, with the aim to identify key features for insecticidal activity, and hopefully find new simplified structures while maintaining the activity. Treating racemic Rocaglamide with a Lewis acid at low temperatures readily generated the benzylic cation at position 8b, which could either be reduced, or trapped by a range of nucleophiles, to give new derivatives in good yields (Scheme 1).

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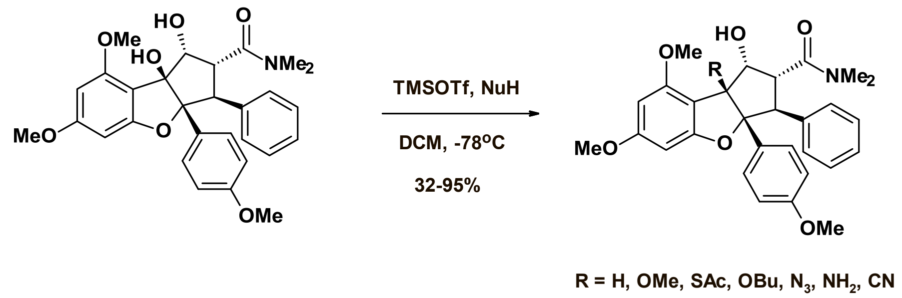
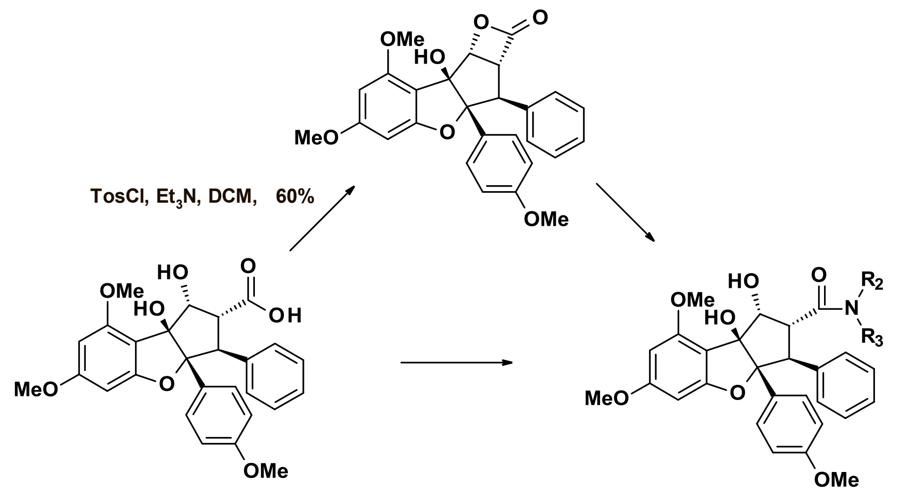
The impact of such modifications on the insecticidal activity was dramatic; all such changes led to a complete loss of biological activity. A number of modifications to position 1 were then investigated, such as inversion of stereochemistry, oxidation to the ketone, oxime formation and reduction of the alcohol. For example, compound **2** could be prepared in 52% yield from compound **1** by treatment with sodium hydride in dimethoxyether with tosyloxychloride. However, all of these modifications led to a loss in insecticidal activity (Table 1).

Modifications to the amide substituent at position 2 were then investigated, with different amides being prepared either from the acid, or through ring opening of the (biologically inactive) lactone (Scheme 2, Table 2).

As can be seen from these results, minor modifications to the dimethyl amide substituent do not greatly impact the insecticidal activity. Racemic compound **6** is quite comparable to the natural product **1**; replacing one or both methyl groups by a hydrogen atom gives compounds **7** and **8** with weaker activity. Replacing the methyl substituent in compound **6** by a hydrogen atom leads again to good levels of activity in compound **9**. Increasing the size of the alkyl group however is not well tolerated, as seen in compounds **11** and **12**; interestingly the morpholine amide **13** shows once again interesting levels of activity, and is clearly better than the piperidine derivative **14**.

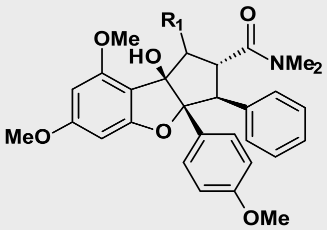


Scheme 1. Modifications at position 8b.



Scheme 2. Amide modifications.

Table 1. Modifications to position 1

COMPOUND	R_1	DOSE ($MG.L^{-1}$) GIVING 80–100% MORTALITY				
		*Heliothis verescens* L1	*Heliothis verescens* L3	*Spodoptera littoralis* L3	*Plutella xylostella* L2-3	*Diabrotica baltealta* L2
1	OH (hashed)	< 3	12.5	12.5	3	3
2	OH (wedged)	100	100	100	100	>100
3	=O	>100	>100	>100	>100	25
4	NHOH (wedged)	>100	>100	>100	100	50
5	H	25	100	100	100	25

Heliothis virescens F – L1 first instar on soybean; L3 third instar on soybean. *Spodoptera littoralis* Boisd – L3 third instar on soybean. *Plutella xylostella* L – second/third instar on cabbage. *Diabrotica baltealta* Lec – L2 second instar on maize seedlings.

Table 2. Amide modifications

COMPOUND	R_2	R_3	DOSE (MG.L^{-1}) GIVING 80–100% MORTALITY				
			Heliothis verescens L1	*Heliothis verescens* L3	*Spodoptera littoralis* L3	*Plutella xylostella* L2-3	*Diabrotica baltealta* L2
1	CH_3	CH_3	< 3	12.5	12.5	3	3
6	CH_3	OCH_3	3	12.5	12.5	25	NT
7	CH_3	H	12.5	25	25	50	NT
8	H	H	3	50	50	25	100
9	H	OCH_3	3	12.5	25	12.5	100
10	CH_3	OH	100	>100	>100	100	1000
11	CH_3	n-C_4H_9	>100	>100	>100	100	3
12	n-C_4H_9	n-C_4H_9	>100	>100	>100	>100	NT
13	-$CH_2CH_2OCH_2CH_2$-		3	50	50	25	100
14	-$(CH_2)_5$-		>100	>100	>100	100	3

NT: not tested

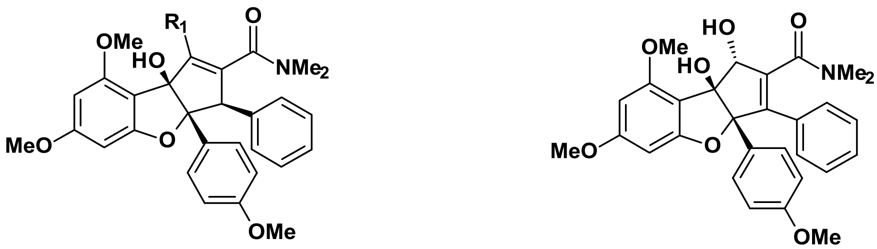
Removal of the stereogenic center at positions 1, 2, or 3 was achieved through incorporation of unsaturation. Indeed, natural products have been isolated[7] where the amide substituent at C-2 is incorporated into a fused 5,6 ring system linked at position 1. Compounds such as **17** could be prepared using an intermediate in the total synthesis published by Trost *et al*.,[11] where a ketone functionality at position 1 could be reduced using tetraethylammonium triacetoxyborohydride. Such unsaturated derivatives however, were essentially inactive against lepidopteran larvae at 100 mgL^{-1} (Fig. 2).

Substituents on the phenyl ring at position 3, together with the *para* methoxy substituent on the 3a phenyl ring in compound **6** were then modified. The impact of such changes on the insecticidal activity compared to compound **6** are shown in Table 3.

Replacement of the methoxy substituent in the phenyl 3a ring by chlorine leads to a slight improvement in the insecticidal activity, as shown with the results for compound **18** compared with compound **6**. Replacement with hydrogen to give compound **19** leads to a reduction in insecticidal activity, and a more dramatic reduction in activity is seen with the phenyl derivative **20**. Maintaining the *para* methoxy substituent on phenyl ring 3a, and introducing a *para* substituent on the phenyl ring 3 results in a dramatic loss of biological activity, as seen with compounds **21**, **22**, and **23**.[12]

We have also reported[13] a total synthesis of the carbocyclic analogue **24** of Rocaglamide, which featured an intramolecular condensation as step 4 to construct the tricyclic skeleton. Introduction of the substituents at positions 8b, 1, and 2 then led to the final compound **24**. This one modification once again had a dramatic effect on the insecticidal activity, with compound **24** being totally inactive as an insecticide (Scheme. 3).

Modifications to the benzofuran phenyl ring were also investigated, either by introducing additional halogen substituents, or by selective modification of the methoxy substituent at position 8. When compared to Rocaglamide (**1**), we observed a dramatic decrease in insecticidal activity when such modifications are made, although the activity against *Plutella xylostella* was maintained with compound **25** (Table 4).



15 R_1 = H

16 R_1 = OMe

17

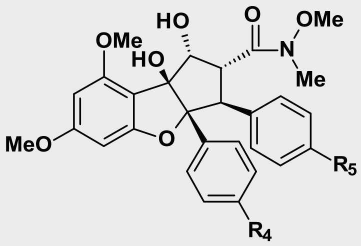
Fig. 2. Unsaturation leads to loss of insecticidal activity.

Impact of Chirality

Another synthetic analogue **32** (Fig. 4) was prepared which displayed comparable activity to Rocaglamide, and offered two points for chemical modification. This compound could be separated into two enantiomers by chiral chromatography to give **32a** and **32b**, which were then compared to the racemate **32** and Rocaglamide (Table 5).

The absolute configurations of **32a** and **32b** were not determined; the assignments have been made based on the insecticidal activity shown in Table 5, where we as-

Table 3. Modifications to substituents on the C-3 and C-3a phenyl rings

COMPOUND	R_4	R_5	DOSE ($MG.L^{-1}$) GIVING 80-100% MORTALITY				
			*Heliothis verescens* L1	*Heliothis verescens* L3	*Spodoptera littoralis* L3	*Plutella xylostella* L2-3	*Diabrotica baltealta* L2
6	OCH_3	H	3	12.5	12.5	25	NT
18	Cl	H	3	3	12.5	3	0.8
19	H	H	25	100	25	25	12.5
20	C_6H_5	H	>100	>100	100	100	NT
21	OCH_3	NH_2	>100	NT	>100*	NT	>100
22	OCH_3	Br	>100	NT	>100*	NT	>100
23	OCH_3	OCH_3	>100	NT	>100*	NT	>100

NT: not tested; * activity against L1 larvae

sume that enantiomer **32b** has the natural configuration.

Enantiomer **32a** proved to be inactive except against *Plutella xylostella* boisd at the highest rate tested (12.5 $mg.L^{-1}$). Interestingly, compound **32b** displayed a rather comparable activity to the racemate **32** and also to Rocaglamide (**1**).

Field Testing

After an intensive synthesis campaign, 3.9 g of racemic Rocaglamide was prepared, which could be separated into both enantiomers using preparative HPLC; details of the separation are shown in Scheme 4.

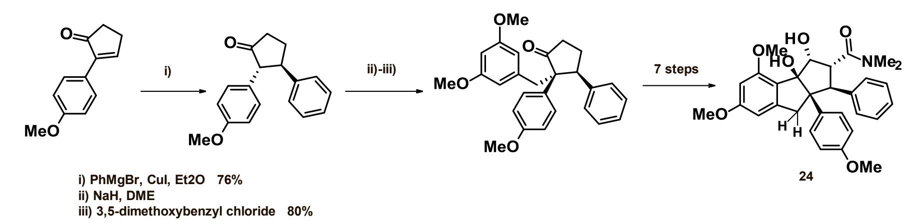
The unnatural enantiomer **33** (biologically inactive) eluted from the column first, followed by the natural enantiomer **34** (**1**). With this quantity of compound **34** in hand, we were keen to test the insecticidal activity in field and semi-field conditions. Four small field trials were conducted with enantiomerically pure synthetic Rocaglamide. In Indonesia, a trial against the rice stemborer at rates of 25 and 10 g active ingredient per hectare (ai/ha) showed no control of this pest. In Thailand, a trial was performed against the diamondback moth in cabbage, with results shown in comparison to a market standard, Abamectin in Table 6.

Although clearly weaker than the standard, some interesting activity was observed at higher rates; however, phytotoxicity to the cabbage plants was also observed. In Egypt, semi-field trials were performed against the Egyptian cotton leaf worm on cotton, but even at the highest rate of 100 g ai/ha, no activity was observed and a phytotoxicity of 50% leaf burn measured. Lastly, a trial in the United States against the cotton bollworm on cotton was performed. Activity was measured five days after application, at a rate of 25 g ai/ha, compared to a market standard Karate (35 g ai/ha). Results are shown in Table 7.

In these two trials, synthetic Rocaglamide showed quite an interesting control of this pest when compared to a market standard Karate.

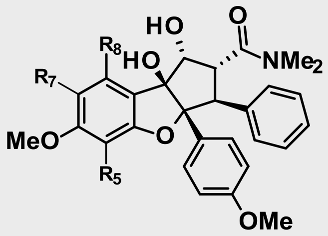
Conclusions

The natural product Rocaglamide is a compelling, but also challenging lead structure for crop protection. In laboratory assays, product-like levels of insecticidal activity against commercially-important lepidopteran pests were observed. Rocaglamide also showed a good duration of activity over time in laboratory assays. There were, however many challenges to face with this natural product program. On the one hand, the insecticidal activity had to be maintained, or even optimized in simplified analogues, whilst simulta-

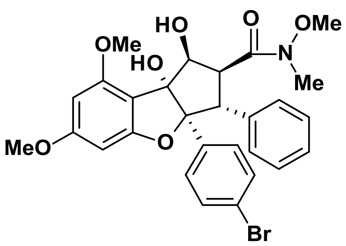


Scheme 3. The carbocyclic analogue **24** of Rocaglamide.

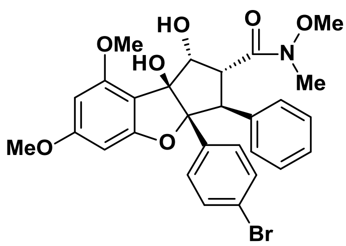
Table 4. Modifications to the benzofuran phenyl ring

COMPOUND	R_5	R_7	R_8	DOSE ($MG.L^{-1}$) GIVING 80-100% MORTALITY				
				*Heliothis verescens* L1	*Heliothis verescens* L3	*Spodoptera littoralis* L3	*Plutella xylostella* L2-3	*Diabrotica baltealta* L2
1	H	H	OCH_3	< 3	12.5	12.5	3	3
25	H	H	O-nBu	>100	>100	>100	12.5	3
26	H	H	H	>100	>100	>100	100	>100
27	H	H	OSO_2CF_3	>100	>100	>100	12.5	25
28	Br	Cl	OCH_3	>100	NT	>100*	NT	>100
29	H	Cl	OCH_3	>100	NT	>100*	NT	>100
30	Cl	H	OCH_3	>100	NT	>100*	NT	>100
31	Br	H	OCH_3	>100	NT	>100*	NT	>100

NT: not tested



32a



32b

Fig. 3. Structures of **32a** and **32b**.

Table 5. Impact of chirality

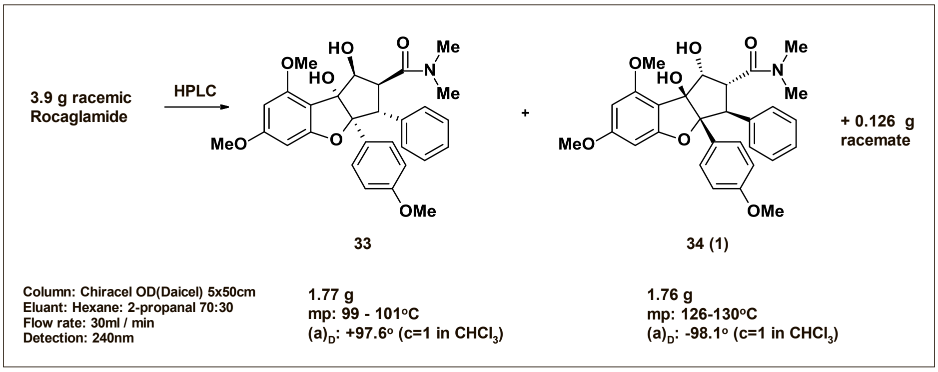
COMPOUND	DOSE ($MG.L^{-1}$) GIVING 80-100% MORTALITY				
	Heliothis verescens L1	*Heliothis verescens* L3	*Spodoptera littoralis* L3	*Plutella xylostella* L2-3	*Diabrotica baltealta* L2
1	< 3	12.5	12.5	3	3
RACEMATE 32	< 3	12.5	3	3	0.2
32a	>12.5	>12.5	>12.5	12.5	>12.5
32b	< 3	12.5	3	3	0.2

neously suppressing the phytotoxicity. In addition, multi-step syntheses with control of many stereochemical centers were a pre-requisite to carefully probe the SAR of synthetic analogues.

The goal to identify simplified analogues of Rocaglamide with equal or improved insecticidal activity could not be achieved. However, two points of modification were identified where structural changes are tolerated and insecticidal activity retained; the dimethyl amide at C-2 could be replaced by N,O-dimethyl hydroxamide, as in compound **6**. Secondly the *para* methoxy substituent on the C-3a phenyl ring could be replaced by halogen (Cl or Br) as seen in compounds **18** and **32**. Chiral separation enabled the testing of both enantiomers of **32** and Rocaglamide, showing chirality to be important.

The mode of action as an insecticide remains unknown. The total lack of activity seen in analogues modified at position 8b, together with the inactive carbocyclic analogue **24** is intriguing. This suggested to us that a carbocation at position 8b might somehow be formed *in vivo*, but this remains pure speculation.

Field trials against four pests in different crops and countries were ultimately disappointing, and somewhat inconclusive, with unacceptable levels of phytotoxicity being observed.



Scheme 4. Chiral separation of racemic Rocaglamide.

Table 6. Results of field trials against *Plutella xylostella* L. in Thailand

Com-pound	% Control at concentration (PPM)				
	25	12.5	6.25	3.125	1.56
34 (1)	67.5	67.5	40	30	22.5
Abamectin	100	100	92.5	77.5	67.5

Table 7. Percent control of *Heliothis virescens* F. in two USA field trials

COMPOUND	Rate	Trial 1	Trial 2	Average
34 (1)	25 g / ha	49.0	73.3	61.2
KARATE	35 g / ha	86.6	87.8	87.2

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