Studies towards the total synthesis of solaneoclepin A: synthesis of analogues containing the tetracyclic left-hand substructure.

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General Introduction

1.1 The Potato Cyst Nematode

Potato cyst nematodes (PCN) are responsible for major losses in the production of industrial, seed and consumption potatoes (Photo 1.1).¹ In 1934 the relationship between these losses and the presence of parasitic nematodes was discovered.

Photo 1.1 The destructive result of PCN

In The Netherlands two species of PCN, i.e. the yellow potato cyst nematode (Globodera rostochiensis) and the white potato cyst nematode (Globodera pallida) (Photo 1.2) are responsible for the damage to potato fields. After in 1948 G. rostochiensis was encountered for the first time, measures were taken to control their further spread. It was
allowed to grow potatoes once every three years on uninfected fields whereas potato cultivation was forbidden on PCN infested fields. These measures were not sufficient to control the PCN and even more drastic actions were taken. Crop rotation, by the use of resistant potato cultivars and by soil fumigation appeared to be a successful strategy to control the yellow PCN.

![Photo 1.2 Globodera pallida; length 463-509 μm, width 19 μm](image)

With the number of yellow PCN on the decline, the amount of white PCN that was encountered increased. It appeared more difficult to develop new PCN resistant potato cultivars so that, chemical treatment became more important. This had a major impact on the environment, eventually leading to governmental interference. The use of chemical crop protection agents and soil fumigants was restricted. Until the nineties *G. pallida* infested soils were usually fumigated every second year, which is currently restricted to every fifth year. As a result, research on more effective and environmentally more acceptable methods to control the PCN was intensified. In 1986, several research groups in The Netherlands initiated research, funded by the Dutch government, to develop novel methods to control the PCN (see: Section 1.2).

The potato cyst nematodes are named after the swollen flask shaped dead bodies of the fertilized female nematodes, the so-called cysts (Photo 1.3). The cyst is a protective covering for the eggs and is resistant to drying and frost. The cyst remains in the soil after the crop has been harvested. A chemical, the so-called hatching agent, given off by the growing potato plant in spring stimulates larvae to hatch from the eggs. The larvae leave the cyst through an opening in the wall and migrate to the host plant. The young juvenile nematodes penetrate the root of the potato plant and live from its fluids, resulting in severe damage to the potato plant. After fertilization, several hundred eggs develop within the female whereupon the female dies, leaving the cyst with the eggs behind, thus closing the live cycle.
It is known that encysted dormant eggs may persist for many years, but as soon as the juvenile nematodes have been hatched, they will die within a period of eight weeks in absence of a source of nutrition. This is considered to be a clue to develop an environmentally friendly method to control the PCN. If one of these hatching agents is applied on an uncultivated potato field it should lead to hatching of the nematodes. Because there is no source of nutrition the juvenile will die from starvation. This principle was validated by field experiments, which showed that the infestation level of infested soil was reduced by treatment with potato plant root extracts, containing the hatching agents.\(^3\)

1.2 Solanoeclepin A

The discovery of a hatching compound produced by the potato plant which could provide a way to combat PCN initiated an extensive research project. In 1986, research groups in the Netherlands cooperated to identify and characterize this hatching agent and to investigate its use as an environmentally benign method to control the PCN. In this research the following partners were involved: LUXAN (Elst), the Netherlands Institute of Carbohydrate Research-TNO (Groningen), TNO-Biotechnology and Chemical Institute (Zeist) and the HLB Agricultural Research Center (Assen). Both TNO divisions were involved in the isolation of the hatching agents while the HLB Agricultural Research Center performed biological activity tests on the samples provided by TNO.

Various samples possessing hatching activity were found. The research then focused on the elucidation of the compound possessing the highest biological activity.\(^4\) This led to the isolation of solanoeclepin A\(^5\) with the systematic name: \textit{trans}-2-(2,13-dihydroxy-9-methoxy-7,7,16-trimethyl-5,10,20-trioxo-19-oxahexacyclo[9.7.0.1\(^3\).6.0\(^5\).8.1\(^{12,15}\).0\(^{12,16}\)]eicosa-1(11),8-dien-15-yl)-cyclopropanecarboxylic acid (Figure 1.1). A total of 0.245 mg of this hatching agent with the molecular formula C\(_{27}\)H\(_{30}\)O\(_9\) and \(M_w = 489.5\) was isolated from one thousand potato plants. The structure elucidation appeared extremely difficult until the natural product
suddenly and unexpectedly crystallized in the NMR tube during attempts to elucidate the 
structure by $^1$H NMR. Consequently, an X-ray crystal structure determination by Schenk and 
coworkers revealed the structure of the natural product. Solanoeclepin A appeared to be an 
extremely active hatching agent. This was illustrated by field experiments, which showed that 
only 0.3 g of the pure compound per hectare is needed to successfully combat PCN.

![Figure 1.1 The crystal structure of solanoeclepin A](image)

The limited information available indicated that the molecule is unstable at pH below 
2 and above 7 and at temperatures above 35 °C. It is not clear which structural features of the 
molecule are responsible for this remarkable base and thermal instability. It is also unknown, 
which structural moieties are responsible for the hatching activity. Probably, the 
cyclobutanone moiety and the methyl enol ether are the centers of enhanced chemical lability, 
which might be revealed by future structure activity relationship (SAR) studies.

1.3 Glycinoeclepin A

Glycinoeclepin A (2, Figure 1.2) is the natural hatching agent of the soybean cyst 
nematode (*Heteropdera glycines*). It was isolated in 1985 from the roots of kidney bean plants 
by Masamune and coworkers. Due to its strong hatch stimulation activity for the soybean 
cyst nematode, it did not take long before total syntheses of this natural product were reported 
in the literature. Furthermore, syntheses of analogues of this compound to gain information 
about the SAR were reported. From this research it was concluded that almost every
structural feature was essential for the hatching activity. Especially, alterations around the hydroxyl group at C-12 and both carboxylic acid groups, were not allowed to maintain a good biological response.

![solanoeclepin A (1) and glycinoeclepin A (2)]

**Figure 1.2 The hatching agents of the potato and soybean cyst nematode**

Comparison of the structures of solanoeclepin A and glycinoeclepin A reveals striking similarities. Although it is known that glycinoeclepin A does not promote the hatching of PCN, the results of its SAR studies could provide valuable information for the directed synthesis of analogues of solanoeclepin A. The synthesis of analogues is necessary since the complex structural features of solanoeclepin A probably not allow an economically attractive synthesis. SAR studies should reveal which parts of the molecule are needed for hatching activity.

### 1.4 Hatching Activity Tests

In order to perform structure activity relationship (SAR) studies, solanoeclepin A and some of its analogues described in this thesis were tested for their biological activity. These hatching activity tests were performed at the HLB Agricultural Research Center, which was formerly located in Assen, but since 2000 in Wijster, The Netherlands. In such activity tests the compounds are added as aqueous solutions (4.5 mL) in concentrations ranging from 1.25 mg to 125 mg per L to an aqueous suspension of ca. 300 nematode eggs in water (0.5 mL). The suspensions are then acidified to pH = 4.0 and incubated at 20 °C for several days. After ten days samples are taken to estimate the number of PCN that have been hatched. The hatching activity is compared with potato root diffuse (PRD), 'hatching agent D' (Standard of 1965) and tap water as a blanco, which are tested simultaneously. Usually PRD affords 80% of PCN hatching at the highest concentration after ten days. Spontaneous hatching, as observed with tap water, is usually about 30%. The number of hatched PCN is a direct indication of the hatching activity of the tested compound.

### 1.5 Synthesis of Solanoeclepin A

Besides the interesting biological activity, solanoeclepin A is a very challenging target for total synthesis. Its unusual skeleton contains nine stereogenic centers and features all ring
sizes ranging from three to seven. A retrosynthetic approach of this unique compound is proposed in Scheme 1.1. Herein, solanoeclepin A (1) is divided into two parts of comparable size and complexity (5 and 6). To obtain these two fragments, the seven-membered ring in solanoeclepin A is disconnected twice. Ring opening between C-6 and C-7 of \( \alpha \)-hydroxyketone 3 is followed by a second detachment between the C-9 and C-19 of compound 4.

\textbf{Scheme 1.1}

\begin{equation}
\begin{array}{c}
\text{1} \\
\text{H} \\
\text{O} \\
\text{O} \\
\text{Me} \\
\text{PG} \\
\text{OH} \\
\text{CO}_2\text{H} \\
\text{3} \\
\text{PG} \\
\text{OH} \\
\text{CO}_2\text{H} \\
\end{array}
\end{equation}

\begin{equation}
\begin{array}{c}
\text{4 (R = H or OEt)} \\
\text{PG} \\
\text{OH} \\
\text{CO}_2\text{H} \\
\text{5} \\
\text{PG} \\
\text{OH} \\
\text{CO}_2\text{H} \\
\text{6} \\
\text{PG} \\
\end{array}
\end{equation}

\text{PG} = \text{protective group}

Solanoeclepin A was expected to be accessible via oxidation of acyloin 3 followed by methylation. The \( \alpha \)-hydroxyketone was expected to arise from an intramolecular acyloin condensation of diester 4 (R = OEt). Possibly, the conditions for this cyclization are not mild enough and alternatives have to be found. An intramolecular McMurry coupling of dialdehyde 4 (R = H) followed by oxidation and methylation might also give the desired compound. Diester 4 (R = OEt) and dialdehyde 4 (R = H) were expected to arise from a chromium-mediated coupling reaction of aldehyde 5 and vinyl triflate 6. However, the stereochemical outcome of this reaction is uncertain. Nevertheless, it should be possible to obtain the correct absolute configuration at C-19 via an oxidation-diastereoselective reduction sequence.

Alternatively, the seven-membered ring may be constructed by a ring-closing metathesis (RCM) reaction of divinyl compound 8 (Scheme 1.2). Oxidative functionalization of the least substituted double bond of diene 7 can possibly afford acyloin 3. Triene 8 was expected to be accessible from the product of a chromium-mediated coupling reaction of vinyl triflate 6 and aldehyde 9. An important advantage of this convergent approach is the simple access to analogues of solanoeclepin A, which are needed for structure activity relationship studies. Combination of different types of aldehydes such as 5 and 9 and simplified analogues of vinyl triflate 6 should lead to a variety of compounds to be tested.
1.6 Purpose and Outline of this Investigation

In this thesis, the enantioselective synthesis of solanoeclepin A is investigated via the preparation of compounds that contain important structural features of this fascinating molecule. A versatile route has been developed to construct the seven-membered ring. This route was used to synthesize solanoeclepin A analogues containing the tetracyclic left-hand substructure of solanoeclepin A (viz. compounds 11 - 13 in Scheme 1.3).¹⁴

In Chapter 2, enantiopure syntheses of aldehydes 5 and 9 are described. A stereoselective intramolecular furan Diels-Alder reaction was used to construct the oxabicycle. After the appropriate functional group transformations the two aldehydes were obtained in good overall yields.

Scheme 1.3

To study the construction of the seven-membered ring, aldehyde 5 was joined to vinyl triflate 10 in Chapter 3. Then, the viability of constructing the seven-membered ring by using
carbonyl coupling reactions, such as the acyloin condensation and the McMurry reaction was investigated.

Because the approach to the formation of the seven-membered ring in Chapter 3 appeared unsuccessful a new synthetic route was developed in Chapter 4. This instance, aldehyde 9 and vinyl triflate 10 were coupled. The seven-membered ring could eventually be closed via a RCM reaction and further functionalization afforded the desired tetracyclic left-hand substructure.

The developed route was expanded through the synthesis of two new substrates (12 and 13) in Chapter 5.

1.7 Acknowledgments

Dr. Ir. A. Mulder is kindly thanked for the photos that are shown in this Chapter.

1.8 References and Notes


2 The most important active compounds in this treatment are methyl isothiocyanate and (Z)-1,3-dichloropropene, see: van Rijn, J. P.; van Straalen, N. M.; Willems, J. *Handboek Bestrijdingsmiddelen, Gebruik & Milieueffecten; deel D, Nematiciden; VU Uitgeverij*, Amsterdam, 1995.


5 This name was chosen to indicate the relationship with the earlier reported structure of the soybean cyst nematode glycinoeclepin A, see ref. 7.


General Introduction


Mulder, A. Personal communication, September 1997.

Cysts (population E447) are soaked in water at 4 °C for 7 days and are crushed to give the nematode eggs that are used in the hatching activity tests.

Three weeks prior to the hatching activity test potato plants are grown. PRD is drawn form the potato plant (ca. 100 mL per 5 liter pot).

