

## Elucidation of the Structure of Solanoclepin A, a Natural Hatching Factor of Potato and Tomato Cyst Nematodes, by Single-crystal X-ray Diffraction\*

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Potato crops can be severely damaged by potato cyst nematodes *Globodera rostochiensis* and *Globodera pallida*, nematodes highly specific to potatoes and some other *Solanaceae*. Hatching of juveniles is controlled by agents excreted by the roots of some *Solanaceae* species. Over seventy years much effort has been expended by many groups to isolate these agents and to determine their structures. However, all attempts have failed. We report here the structure determination of a hatching factor excreted from potato and tomato roots. The hatching factor bears some resemblance to Glycinoclepins as found by Masamune *et al.*<sup>2–5</sup> and is hence designated as Solanoclepin A.<sup>1</sup>

C<sub>27</sub>H<sub>30</sub>O<sub>9</sub>·3H<sub>2</sub>O,  $M_r = 498.5$ , monoclinic,  $P2_1$ ,  $a = 11.289(2)$ ,  $b = 20.644(4)$ ,  $c = 11.632(12)$  Å,  $\beta = 90.81(4)$ ,  $V = 2711(3)$  Å<sup>3</sup>,  $Z = 4$ ,  $D_x =$

\* Dedicated to Professor Boris Kamenar on the occasion of his 70<sup>th</sup> birthday.

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1.35 g cm<sup>-3</sup>,  $\lambda(\text{Cu-K}\alpha) = 1.5418 \text{ \AA}$ ,  $\mu(\text{Cu-K}\alpha) = 9.0 \text{ cm}^{-1}$ ,  $F(000) = 1176$ ,  $-60 \text{ }^\circ\text{C}$ . Final  $R = 0.117$  for 3721 observed reflections.

*Key words:* crystal structure determination, natural compound, potato roots.

## INTRODUCTION

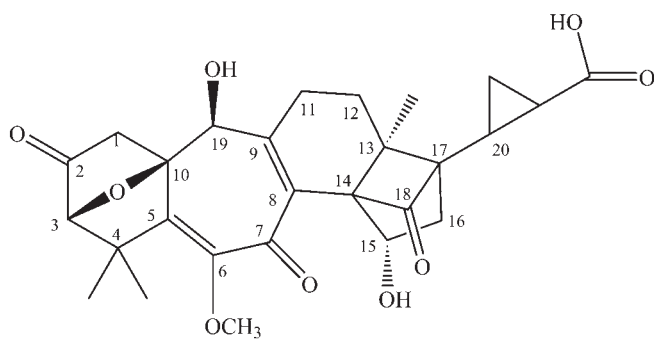
Potato cyst nematodes (*Globodera rostochiensis* and *Globodera pallida*) are responsible for major losses in the production of industrial, seed and consumption potatoes. The potential threat to the environment of considerable quantities of systemic and fumigant nematicides used for control has triggered attempts to isolate the hatching factors of *G. rostochiensis* and *G. pallida* in order to apply these factors to soil while it is not cultivated. In this way, the nematodes could be hatched and would die in the absence of host plants. However, the isolation and structure elucidation of the hatching factors of *G. rostochiensis* and *G. pallida* were hampered by the extremely low quantities excreted by potato roots, by the absence of a good bioassay and by the presence of inhibitors in the root exudates.

Already in 1923, Baunacke<sup>6</sup> understood that host root exudates from potato plants play an active role in the hatching process of potato cyst nematodes. O'Brien and Prentice<sup>7</sup> reported the hatch-inducing activity of potato root exudates on the potato cyst nematode whereas Triffith<sup>8</sup> was the first to investigate in detail the excretions of hatching factors by potato roots. Many groups across the world have been intrigued since by the behaviour of hatching factors of cyst nematodes and many have tried to solve their structures (Janzen and van der Tuin,<sup>9</sup> Calam *et al.*,<sup>10</sup> Marrian *et al.*,<sup>11</sup> Clarke,<sup>12</sup> Atkinson *et al.*).<sup>13</sup> In this paper, a successful structure determination of the hatching factor of *G. rostochiensis* and *G. pallida* is reported.

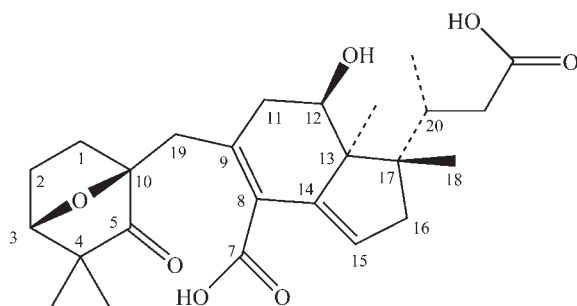
The resemblance to Glycinoeclepin A, the hatching factor for soybean cyst nematodes, is striking (see Scheme I)<sup>14</sup>.

## EXPERIMENTAL DETAILS AND RESULTS

After extensive research, 200  $\mu\text{g}$  of pure compound was purified from crude biological material obtained from a potato cultivation. When this small amount was subjected to NMR experiments, the compound crystallised overnight into 10 crystals of approximately equal size and generally suitable for single-crystal X-ray diffraction. The first two crystals we tried were unstable and a twin, respectively. The third one, an irregularly shaped crystal with approximate dimensions  $0.5 \times 0.6 \times 0.8 \text{ mm}$ , was used for data



Solanoeclepin A



Glycinoeclepin A

Scheme I. Comparison of Solanoeclepin A with Glycinoeclepin A.

collection on an Enraf-Nonius CAD-4 diffractometer with graphite-monochromated Cu-K $\alpha$  radiation and  $\omega$ - $2\theta$  scan. Details of the data collection and structure refinement are listed in Table I.

All attempts to solve the structure by all conventional Direct Methods packages failed, also due to the fact that we had no knowledge about the nature of the compound we were looking for. The structure was finally solved by the program package CRUNCH (de Gelder *et al.*, 1993).<sup>15</sup>

The CRUNCH procedure uses Direct Methods based on Karle-Hauptman determinants in combination with advanced Fourier recycling methods. Default settings of CRUNCH were used to *ab initio* solve the structure to a final *R*-value (based on *E*'s) of 0.058. 10 Karle-Hauptman matrices of order 39 were constructed by the Direct Method program DETER using an estimated number of non-hydrogen atoms in the cell of 160. The determinants of the matrices were concurrently refined as a function of the phases

TABLE I  
Crystal data and structure refinement

Empirical formula	$C_{27}H_{30}O_9 \cdot 3H_2O$
Formula weight	489.5
Radiation, $\lambda / \text{\AA}$	Cu-K $\alpha$ , 1.5418
Crystal system	monoclinic
Space group	$P2_1$
Unit cell dimensions / $\text{\AA}$ , deg	$a = 11.289(2)$ $b = 20.644(4)$ $c = 11.632(12)$ $\beta = 90.81(4)$
Volume / $\text{\AA}^3$	2711(3)
$Z$	4
Density (calculated) / $\text{g cm}^{-3}$	1.35
$\mu / \text{cm}^{-1}$	9.0
$F(000)$	1176
Crystal size / mm	$0.5 \times 0.6 \times 0.8$
$\theta$ range / $^\circ$	4–70
Index ranges	$-11 \leq h \leq 0$ $0 \leq k \leq 29$ $-10 \leq l \leq 11$
Collected reflections	5237
Independent reflections	5237
Reference reflections (hourly)	$\bar{2} 0 0, 0 \bar{2} \bar{1}$
Decay	10%
Observed reflections	3721 [ $I_o > 2.5\sigma(I_o)$ ]
Weighting scheme	$1/w = 6.8 + 0.011 * (\sigma(F_{\text{obs}}))^2 + 10^{-4} / \sigma(F_{\text{obs}})$
Data/restraints/parameters	3721/139/919
Goodness of fit	0.93
$\Delta / \sigma_{\text{max}}$	0.77
Residual el. density ( $e / \text{\AA}^3$ )	-0.49, 0.50
$R$	0.117 <sup>a</sup>
$R_w$	0.127 <sup>b</sup>

$$^a R = \Sigma |F_o| - |F_c| / \Sigma |F_o|$$

$$^b R_w = \{\Sigma [w(F_o^2 - F_c^2)^2] / \Sigma [w(F_o^2)^2]\}^{1/2}$$

which were initially set at random values. Twenty trial starting sets were needed to obtain a molecular fragment of 33 peaks from which the AUTO-FOUR program was able to complete the structure.

The asymmetric part of the unit cell contained two independent identical molecules of Solanoclepin A. The hydrogen atoms were calculated. After isotropic refinement of this initial model, a  $\Delta F$  synthesis revealed six peaks, which were interpreted as six water molecules. Full-matrix least-squares refinement on  $F$ , anisotropic for the non-hydrogen atoms and isotropic for the hydrogen atoms, restraining the latter in such a way that the distance to their carrier remained constant at approximately 1.0 Å and with a constant temperature factor of  $U = 0.11 \text{ \AA}^2$  converged to  $R = 0.117$  (the data have been deposited at the Cambridge Crystallographic Data Centre under no. 107997).

An empirical absorption correction was applied with coefficients in the range of 0.75–1.19 (DIFABS: Walker and Stuart, 1983).<sup>16</sup> The secondary isotropic extinction coefficient (Zachariasen 1967; Larson 1969)<sup>17,18</sup> refined to  $g = 1.8(3) \times 10^3$ . Scattering factors were taken from Cromer and Mann (1968);<sup>19</sup> International Tables for X-ray Crystallography (1974).<sup>20</sup> All calculations were performed with XTAL (Hall, King and Stewart 1995),<sup>21</sup> unless stated otherwise. The relatively high  $R$ -factors reflect the rather poor quality of the crystals available and of which the best was used for data collection. Details of the molecular structure found by X-ray diffraction were confirmed by <sup>1</sup>H NMR, Fourier IR and mass spectrometry and also by the resemblance to the hatching factor for soybean cyst nematodes.

A PLUTO (Motherwell and Clegg, 1983)<sup>22</sup> plot of one of the two molecules (A) is depicted in Figure 1.

The six water molecules form a network of hydrogen bridges between the two molecules; a schematic representation is shown in Figure 2 and the numerical values are listed in Table V.

Matching the two molecules led to RMS = 0.82 Å (hydrogen atoms were not taken into account), the greatest differences occurring at the cyclopropane end of the molecules (leaving these ends led to RMS = 0.12 Å). Hatching factors produced by tomato plants are as effective in inducing hatch in potato cyst nematodes as those from potato plants. This gave ground to the assumption that the tomato and potato hatching factors are similar, if not identical.<sup>9</sup> After elucidation of the structure of the hatching factor of potato cyst nematodes, also the hatching factor from tomato root exudates could be isolated with the same procedure and gave a product with <sup>1</sup>H NMR spectrum identical to the structure of the hatching factor from potatoes.

To our knowledge, this is the first structure that contains both three-, four-, five-, six- and seven-membered rings: this feature has been checked in

TABLE II

Fractional coordinates of the non-hydrogen atoms and equivalent isotropic thermal parameters

Atom	$x$	$y$	$z$	$U_{\text{eq}}$
C(1a)	1.136(2)	0.6698(9)	0.641(2)	0.04(1)
C(2a)	1.253(2)	0.694(1)	0.674(2)	0.05(1)
C(3a)	1.294(2)	0.743(1)	0.580(2)	0.05(1)
C(4a)	1.328(2)	0.701(1)	0.478(2)	0.05(1)
C(5a)	1.212(2)	0.670(1)	0.440(2)	0.05(1)
C(6a)	1.192(1)	0.624(1)	0.365(1)	0.040(9)
C(7a)	1.075(1)	0.5941(9)	0.337(1)	0.037(9)
C(8a)	0.971(1)	0.5922(9)	0.421(1)	0.031(8)
C(9a)	0.937(1)	0.6440(9)	0.485(1)	0.030(8)
C(10a)	1.118(2)	0.7018(9)	0.521(2)	0.04(1)
C(11a)	0.826(1)	0.638(1)	0.563(1)	0.04(1)
C(12a)	0.750(2)	0.577(1)	0.548(2)	0.04(1)
C(13a)	0.828(1)	0.5163(8)	0.535(1)	0.035(8)
C(14a)	0.917(2)	0.526(1)	0.432(1)	0.038(9)
C(15a)	0.986(2)	0.4648(9)	0.420(2)	0.038(9)
C(16a)	0.883(2)	0.413(1)	0.435(2)	0.06(1)
C(17a)	0.777(2)	0.460(1)	0.466(2)	0.05(1)
C(18a)	0.810(2)	0.5051(9)	0.357(1)	0.05(1)
C(19a)	0.992(1)	0.706(1)	0.479(1)	0.038(9)
C(20a)	0.665(2)	0.424(1)	0.493(2)	0.05(1)
C(21a)	0.580(2)	0.406(1)	0.396(3)	0.08(2)
C(22a)	0.553(1)	0.462(1)	0.484(2)	0.05(1)
C(23a)	0.460(1)	0.447(1)	0.574(2)	0.06(1)
C(28a)	0.887(2)	0.502(1)	0.655(2)	0.06(1)
C(29a)	1.431(2)	0.655(1)	0.500(2)	0.06(1)
C(30a)	1.366(2)	0.750(1)	0.379(2)	0.07(1)
C(31a)	1.315(2)	0.539(1)	0.330(2)	0.08(2)
O(1a)	1.173(1)	0.7648(8)	0.544(1)	0.044(7)
O(2a)	1.313(1)	0.6801(8)	0.762(1)	0.050(8)
O(3a)	1.280(1)	0.6045(8)	0.298(1)	0.050(7)
O(4a)	1.063(1)	0.5669(8)	0.2466(9)	0.044(7)
O(5a)	1.0862(9)	0.4629(8)	0.496(1)	0.042(7)
O(6a)	0.754(1)	0.5197(8)	0.272(1)	0.062(9)
O(7a)	0.991(1)	0.7343(8)	0.369(1)	0.043(7)
O(8a)	0.438(1)	0.3972(9)	0.614(2)	0.08(1)
O(9a)	0.406(2)	0.5006(9)	0.608(1)	0.08(1)
C(1b)	0.808(1)	0.686(1)	0.144(2)	0.05(1)

TABLE II (cont.)

Atom	$x$	$y$	$z$	$U_{eq}$
C(2b)	0.688(2)	0.662(1)	0.165(2)	0.05(1)
C(3b)	0.649(2)	0.625(1)	0.057(2)	0.05(1)
C(4b)	0.615(1)	0.672(1)	-0.034(2)	0.04(1)
C(5b)	0.740(1)	0.696(1)	-0.063(1)	0.04(1)
C(6b)	0.777(2)	0.739(1)	-0.139(2)	0.04(1)
C(7b)	0.898(2)	0.764(1)	-0.161(1)	0.039(9)
C(8b)	0.993(2)	0.7601(9)	-0.070(2)	0.04(1)
C(9b)	1.020(1)	0.7075(9)	-0.009(1)	0.027(8)
C(10b)	0.825(2)	0.6583(9)	0.016(1)	0.041(9)
C(11b)	1.117(1)	0.705(1)	0.079(1)	0.035(8)
C(12b)	1.204(2)	0.763(1)	0.075(2)	0.04(1)
C(13b)	1.136(1)	0.8271(9)	0.061(1)	0.035(9)
C(14b)	1.062(1)	0.8218(9)	-0.056(1)	0.026(7)
C(15b)	1.009(2)	0.888(1)	-0.069(1)	0.04(1)
C(16b)	1.110(2)	0.936(1)	-0.031(2)	0.06(1)
C(17b)	1.210(1)	0.8814(9)	-0.005(1)	0.037(9)
C(18b)	1.189(2)	0.838(1)	-0.115(1)	0.05(1)
C(19b)	0.951(1)	0.6447(9)	-0.018(1)	0.032(8)
C(20b)	1.334(2)	0.898(1)	0.043(2)	0.05(1)
C(21b)	1.348(2)	0.953(2)	0.119(2)	0.08(2)
C(22b)	1.390(1)	0.956(1)	-0.002(2)	0.05(1)
C(23b)	1.522(1)	0.956(1)	-0.012(2)	0.05(1)
C(28b)	1.083(2)	0.847(1)	0.175(2)	0.05(1)
C(29b)	0.528(2)	0.722(1)	-0.003(3)	0.11(2)
C(30b)	0.572(2)	0.632(1)	-0.145(2)	0.09(2)
C(31b)	0.659(2)	0.836(1)	-0.191(2)	0.07(1)
O(1b)	0.759(1)	0.5965(7)	0.028(1)	0.040(6)
O(2b)	0.632(1)	0.6734(9)	0.251(1)	0.08(1)
O(3b)	0.688(1)	0.7666(9)	-0.214(1)	0.061(9)
O(4b)	0.912(1)	0.7953(8)	-0.245(1)	0.050(8)
O(5b)	0.905(1)	0.8965(8)	-0.009(1)	0.056(8)
O(6b)	1.233(1)	0.8222(9)	-0.195(1)	0.062(9)
O(7b)	0.950(1)	0.6219(8)	-0.134(1)	0.048(7)
O(8b)	1.566(1)	0.9964(8)	-0.087(1)	0.058(8)
O(9b)	1.586(1)	0.925(1)	0.053(2)	0.08(1)
O(1w)	1.199(1)	0.5133(7)	0.069(1)	0.041(7)
O(2w)	1.187(1)	0.5784(8)	0.862(1)	0.049(8)
O(3w)	1.214(1)	0.3580(9)	0.437(1)	0.07(1)
O(4w)	1.234(1)	0.3617(9)	0.823(1)	0.08(1)
O(5w)	1.195(1)	0.4766(9)	0.709(1)	0.065(9)

TABLE III

Bond distances of the non-hydrogen atoms / Å with standard deviations in parentheses

C(1a)–C(2a)	1.46(3)	C(1b)–C(2b)	1.47(3)
C(1a)–C(10a)	1.56(3)	C(1b)–C(10b)	1.61(3)
C(2a)–C(3a)	1.57(3)	C(2b)–C(3b)	1.53(3)
C(2a)–O(2a)	1.26(2)	C(2b)–O(2b)	1.22(3)
C(3a)–C(4a)	1.51(3)	C(3b)–C(4b)	1.48(3)
C(3a)–O(1a)	1.50(2)	C(3b)–O(1b)	1.41(2)
C(4a)–C(5a)	1.51(3)	C(4b)–C(5b)	1.54(2)
C(4a)–C(29a)	1.53(3)	C(4b)–C(29b)	1.48(3)
C(4a)–C(30a)	1.59(3)	C(4b)–C(30b)	1.60(3)
C(5a)–C(6a)	1.31(3)	C(5b)–C(6b)	1.33(3)
C(5a)–C(10a)	1.58(3)	C(5b)–C(10b)	1.53(3)
C(6a)–C(7a)	1.50(2)	C(6b)–C(7b)	1.48(3)
C(6a)–O(3a)	1.33(2)	C(6b)–O(3b)	1.44(2)
C(7a)–C(8a)	1.54(2)	C(7b)–C(8b)	1.49(2)
C(7a)–O(4a)	1.19(2)	C(7b)–O(4b)	1.19(2)
C(8a)–C(9a)	1.36(2)	C(8b)–C(9b)	1.33(3)
C(8a)–C(14a)	1.49(3)	C(8b)–C(14b)	1.50(3)
C(9a)–C(11a)	1.56(2)	C(9b)–C(11b)	1.49(2)
C(9a)–C(19a)	1.43(3)	C(9b)–C(19b)	1.52(2)
C(10a)–C(19a)	1.50(2)	C(10b)–C(19b)	1.51(2)
C(10a)–O(1a)	1.46(2)	C(10b)–O(1b)	1.49(2)
C(11a)–C(12a)	1.53(3)	C(11b)–C(12b)	1.54(3)
C(12a)–C(13a)	1.53(3)	C(12b)–C(13b)	1.55(3)
C(13a)–C(14a)	1.59(2)	C(13b)–C(14b)	1.59(2)
C(13a)–C(17a)	1.53(3)	C(13b)–C(17b)	1.60(2)
C(13a)–C(28a)	1.56(2)	C(13b)–C(28b)	1.52(2)
C(14a)–C(15a)	1.50(3)	C(14b)–C(15b)	1.51(3)
C(14a)–C(18a)	1.54(3)	C(14b)–C(18b)	1.63(2)
C(15a)–C(16a)	1.58(3)	C(15b)–C(16b)	1.56(3)
C(15a)–O(5a)	1.43(2)	C(15b)–O(5b)	1.40(2)
C(16a)–C(17a)	1.58(3)	C(16b)–C(17b)	1.61(3)
C(17a)–C(18a)	1.62(3)	C(17b)–C(18b)	1.57(3)
C(17a)–C(20a)	1.51(3)	C(17b)–C(20b)	1.53(3)
C(18a)–O(6a)	1.20(2)	C(18b)–O(6b)	1.12(2)
C(19a)–O(7a)	1.41(2)	C(19b)–O(7b)	1.43(2)
C(20a)–C(21a)	1.51(3)	C(20b)–C(21b)	1.45(4)
C(20a)–C(22a)	1.50(3)	C(20b)–C(22b)	1.45(3)
C(21a)–C(22a)	1.58(4)	C(21b)–C(22b)	1.49(3)
C(22a)–C(23a)	1.53(3)	C(22b)–C(23b)	1.50(2)
C(23a)–O(8a)	1.15(3)	C(23b)–O(8b)	1.31(3)
C(23a)–O(9a)	1.33(3)	C(23b)–O(9b)	1.22(3)



TABLE IV

Bond angles/ $^{\circ}$  of the non-hydrogen atoms with standard deviations in parentheses

C(2a)–C(1a)–C(10a)	101(1)	C(2b)–C(1b)–C(10b)	99(2)
C(1a)–C(2a)–C(3a)	108(2)	C(1b)–C(2b)–C(3b)	107(2)
C(1a)–C(2a)–O(2a)	128(2)	C(1b)–C(2b)–O(2b)	124(2)
C(3a)–C(2a)–O(2a)	124(2)	C(3b)–C(2b)–O(2b)	129(2)
C(2a)–C(3a)–C(4a)	105(2)	C(2b)–C(3b)–C(4b)	109(2)
C(2a)–C(3a)–O(1a)	96(1)	C(2b)–C(3b)–O(1b)	99(1)
C(4a)–C(3a)–O(1a)	101(1)	C(4b)–C(3b)–O(1b)	109(2)
C(3a)–C(4a)–C(5a)	104(2)	C(3b)–C(4b)–C(5b)	98(1)
C(3a)–C(4a)–C(29a)	115(2)	C(3b)–C(4b)–C(29b)	117(2)
C(3a)–C(4a)–C(30a)	107(2)	C(3b)–C(4b)–C(30b)	108(2)
C(5a)–C(4a)–C(29a)	116(2)	C(5b)–C(4b)–C(29b)	116(2)
C(5a)–C(4a)–C(30a)	107(2)	C(5b)–C(4b)–C(30b)	105(2)
C(29a)–C(4a)–C(30a)	107(2)	C(29b)–C(4b)–C(30b)	111(2)
C(4a)–C(5a)–C(6a)	130(2)	C(4b)–C(5b)–C(6b)	132(2)
C(4a)–C(5a)–C(10a)	104(2)	C(4b)–C(5b)–C(10b)	106(2)
C(6a)–C(5a)–C(10a)	126(2)	C(6b)–C(5b)–C(10b)	123(2)
C(5a)–C(6a)–C(7a)	126(2)	C(5b)–C(6b)–C(7b)	130(2)
C(5a)–C(6a)–O(3a)	119(2)	C(5b)–C(6b)–O(3b)	117(2)
C(7a)–C(6a)–O(3a)	114(2)	C(7b)–C(6b)–O(3b)	113(2)
C(6a)–C(7a)–C(8a)	123(1)	C(6b)–C(7b)–C(8b)	121(1)
C(6a)–C(7a)–O(4a)	118(1)	C(6b)–C(7b)–O(4b)	118(2)
C(8a)–C(7a)–O(4a)	118(2)	C(8b)–C(7b)–O(4b)	120(2)
C(7a)–C(8a)–C(9a)	123(2)	C(7b)–C(8b)–C(9b)	125(2)
C(7a)–C(8a)–C(14a)	113(1)	C(7b)–C(8b)–C(14b)	114(2)
C(9a)–C(8a)–C(14a)	124(1)	C(9b)–C(8b)–C(14b)	121(1)
C(8a)–C(9a)–C(11a)	119(2)	C(8b)–C(9b)–C(11b)	124(2)
C(8a)–C(9a)–C(19a)	124(1)	C(8b)–C(9b)–C(19b)	123(1)
C(11a)–C(9a)–C(19a)	117(2)	C(11b)–C(9b)–C(19b)	113(1)
C(1a)–C(10a)–C(5a)	106(1)	C(1b)–C(10b)–C(5b)	107(1)
C(1a)–C(10a)–C(19a)	115(1)	C(1b)–C(10b)–C(19b)	116(1)
C(1a)–C(10a)–O(1a)	99(1)	C(1b)–C(10b)–O(1b)	99(1)
C(5a)–C(10a)–C(19a)	118(1)	C(5b)–C(10b)–C(19b)	122(1)
C(5a)–C(10a)–O(1a)	101(1)	C(5b)–C(10b)–O(1b)	101(1)
C(19a)–C(10a)–O(1a)	114(2)	C(19b)–C(10b)–O(1b)	110(1)
C(9a)–C(11a)–C(12a)	117(2)	C(9b)–C(11b)–C(12b)	115(2)
C(11a)–C(12a)–C(13a)	111(1)	C(11b)–C(12b)–C(13b)	110(1)
C(12a)–C(13a)–C(14a)	110(1)	C(12b)–C(13b)–C(14b)	107(1)

(continued)

TABLE IV (cont.)

C(12a)–C(13a)–C(17a)	117(1)	C(12b)–C(13b)–C(17b)	113(1)
C(12a)–C(13a)–C(28a)	108(1)	C(12b)–C(13b)–C(28b)	110(1)
C(14a)–C(13a)–C(17a)	86(1)	C(14b)–C(13b)–C(17b)	85(1)
C(14a)–C(13a)–C(28a)	116(1)	C(14b)–C(13b)–C(28b)	124(1)
C(17a)–C(13a)–C(28a)	119(2)	C(17b)–C(13b)–C(28b)	116(2)
C(8a)–C(14a)–C(13a)	116(1)	C(8b)–C(14b)–C(13b)	115(1)
C(8a)–C(14a)–C(15a)	124(1)	C(8b)–C(14b)–C(15b)	124(1)
C(8a)–C(14a)–C(18a)	122(1)	C(8b)–C(14b)–C(18b)	126(1)
C(13a)–C(14a)–C(15a)	107(1)	C(13b)–C(14b)–C(15b)	103(1)
C(13a)–C(14a)–C(18a)	84(1)	C(13b)–C(14b)–C(18b)	84(1)
C(15a)–C(14a)–C(18a)	96(1)	C(15b)–C(14b)–C(18b)	96(1)
C(14a)–C(15a)–C(16a)	100(1)	C(14b)–C(15b)–C(16b)	105(1)
C(14a)–C(15a)–O(5a)	112(1)	C(14b)–C(15b)–O(5b)	113(1)
C(16a)–C(15a)–O(5a)	119(2)	C(16b)–C(15b)–O(5b)	114(2)
C(15a)–C(16a)–C(17a)	100(2)	C(15b)–C(16b)–C(17b)	97(2)
C(13a)–C(17a)–C(16a)	108(2)	C(13b)–C(17b)–C(16b)	102(1)
C(13a)–C(17a)–C(18a)	84(1)	C(13b)–C(17b)–C(18b)	85(1)
C(13a)–C(17a)–C(20a)	126(2)	C(13b)–C(17b)–C(20b)	118(1)
C(16a)–C(17a)–C(18a)	89(1)	C(16b)–C(17b)–C(18b)	98(1)
C(16a)–C(17a)–C(20a)	112(2)	C(16b)–C(17b)–C(20b)	123(2)
C(18a)–C(17a)–C(20a)	131(2)	C(18b)–C(17b)–C(20b)	123(2)
C(14a)–C(18a)–C(17a)	85(1)	C(14b)–C(18b)–C(17b)	84(1)
C(14a)–C(18a)–O(6a)	142(2)	C(14b)–C(18b)–O(6b)	134(2)
C(17a)–C(18a)–O(6a)	131(2)	C(17b)–C(18b)–O(6b)	142(2)
C(9a)–C(19a)–C(10a)	110(2)	C(9b)–C(19b)–C(10b)	108(1)
C(9a)–C(19a)–O(7a)	114(1)	C(9b)–C(19b)–O(7b)	110(1)
C(10a)–C(19a)–O(7a)	108(1)	C(10b)–C(19b)–O(7b)	108(1)
C(17a)–C(20a)–C(21a)	120(2)	C(17b)–C(20b)–C(21b)	119(2)
C(17a)–C(20a)–C(22a)	116(2)	C(17b)–C(20b)–C(22b)	117(2)
C(21a)–C(20a)–C(22a)	63(2)	C(21b)–C(20b)–C(22b)	62(2)
C(20a)–C(21a)–C(22a)	58(1)	C(20b)–C(21b)–C(22b)	59(2)
C(20a)–C(22a)–C(21a)	59(1)	C(20b)–C(22b)–C(21b)	59(2)
C(20a)–C(22a)–C(23a)	115(2)	C(20b)–C(22b)–C(23b)	118(2)
C(21a)–C(22a)–C(23a)	116(2)	C(21b)–C(22b)–C(23b)	114(2)
C(22a)–C(23a)–O(8a)	128(2)	C(22b)–C(23b)–O(8b)	116(2)
C(22a)–C(23a)–O(9a)	110(2)	C(22b)–C(23b)–O(9b)	122(2)
O(8a)–C(23a)–O(9a)	122(2)	O(8b)–C(23b)–O(9b)	122(2)
C(3a)–O(1a)–C(10a)	99(1)	C(3b)–O(1b)–C(10b)	96(1)
C(6a)–O(3a)–C(31a)	110(2)	C(6b)–O(3b)–C(31b)	115(1)

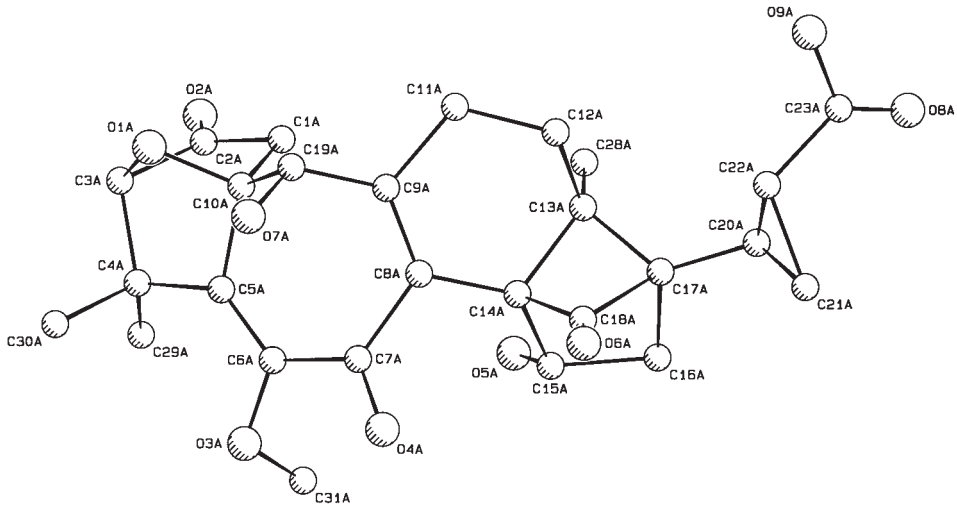


Figure 1. A PLUTO plot of molecule A; hydrogen atoms are left out for clarity.

the Cambridge Crystallographic Database.<sup>23</sup> The corresponding molecular configuration as depicted in Scheme I has some structural relationship with

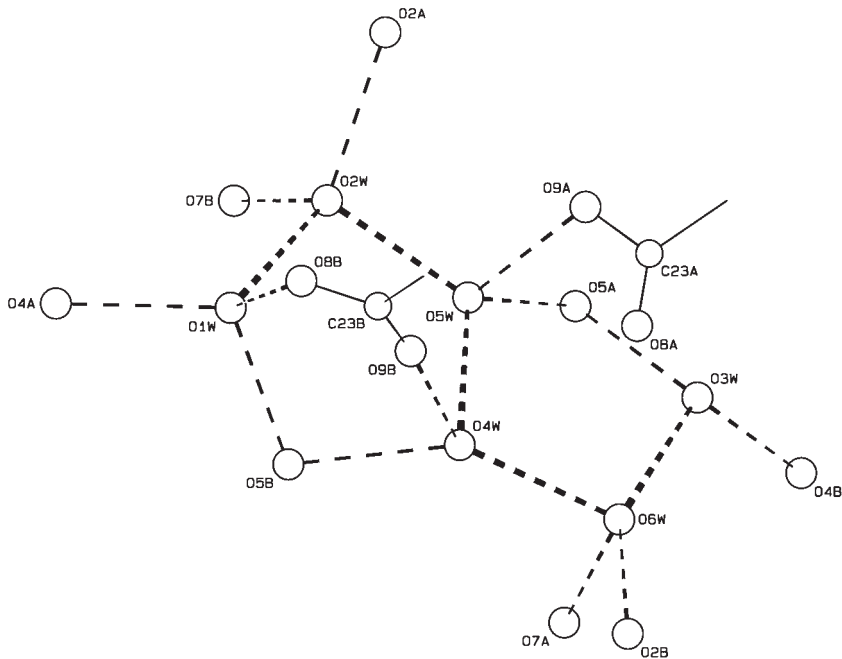


Figure 2. A schematic representation of hydrogen bridges.

TABLE V  
 Numerical values of hydrogen bonds / Å, deg

Donor	H	Acceptor	D–H	H...A	D...A	D–H...A
O(5A)	H(5A)	O(3W)	1.0(2)	1.8(2)	2.69(2)	143(15)
O(5B)	H(5B)	O(1W)	1.0(2)	1.9(2)	2.77(2)	148(15)
O(7A)	H(7A)	O(6W)	1.0(2)	2.2(2)	2.86(2)	123(13)
O(7B)	H(7B)	O(2W)	1.0(2)	2.0(2)	2.83(2)	139(14)
O(9A)	H(9A)	O(5W)	1.0(2)	1.8(2)	2.72(2)	146(15)
O(1W)	H(11W)	O(4A)	1.0(2)	2.1(2)	2.82(2)	130(14)
O(1W)	H(12W)	O(8B)	1.0(2)	2.0(2)	2.68(2)	120(15)
O(1W)	H(12W)	O(2W)	1.0(2)	2.4(2)	2.76(2)	101(11)
O(2W)	H(21W)	O(2A)	1.0(2)	1.8(2)	2.80(2)	172(18)
O(2W)	H(22W)	O(5W)	1.0(1)	1.9(2)	2.76(2)	147(18)
O(3W)	H(31W)	O(8A)	1.0(1)	2.4(2)	3.33(2)	170(16)
O(3W)	H(32W)	O(3B)	1.0(2)	2.5(2)	3.40(2)	152(15)
O(3W)	H(32W)	O(4B)	1.0(2)	2.1(2)	2.94(2)	138(15)
O(4W)	H(41W)	O(5B)	1.0(2)	2.4(2)	2.78(2)	100(15)
O(4W)	H(41W)	O(9B)	1.0(2)	1.9(2)	2.80(2)	141(16)
O(4W)	H(42W)	O(5W)	1.0(2)	1.8(2)	2.75(3)	150(17)
O(5W)	H(51W)	O(5A)	1.0(2)	1.8(2)	2.76(2)	160(16)
O(5W)	H(52W)	O(2W)	1.0(2)	1.9(2)	2.76(2)	144(18)
O(6W)	H(61W)	O(7A)	1.0(1)	2.0(1)	2.86(2)	137(16)
O(6W)	H(62W)	O(4W)	1.0(11)	2.2(7)	2.82(2)	123(13)
O(6W)	H(62W)	O(8A)	1.0(11)	2.5(1)	3.29(2)	138(12)

Glycinoeclepins. Remarkable differences between these structures are the presence of the conjugated heptadiene – one structure in ring B, the butanone structure in ring D and the cyclopropane group in the side chain of the hatching factor of potato cyst nematodes. Although attempts to determine the correct enantiomorph failed, comparison of the present compound with the natural hatching factor for the soybean cyst nematodes<sup>2–5</sup> makes it plausible that the enantiomer presented here is the right one.

Although Glycinoeclepin A does not hatch *G. rostochiensis* and *G. pallida*, it seems appropriate to express the similarity with Glycinoeclepin A in the biological function as well as in the structure in the assignment of the trivial name Solanoeclepin A to the isolated hatching factor of potatoes and

tomatoes. Actually Solanoeclepin A can be considered as a tetranortriterpene derived from gonanane with the systematic name: *trans*-2-[9 $\alpha$ ,15-dihydroxy-6-methoxy-4,4,13-trimethyl-2,7-dioxo-14,17-carbonyl-3,10-epoxy-B(9 $\alpha$ )-homo(13)-gona-5,18-dien-17yl]cyclopropane carboxylic acid.

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### SAŽETAK

#### **Razjašnjenje strukture solanoeklepina A, prirodnog faktora razvoja cističnih nematoda krumpira i rajčice, difrakcijom rentgenskih zraka na jediničnom kristalu**

*Henk Schenk, René A. J. Driessen, René de Gelder, Kees Goubitz, Heiko Nieboer, Ingrid E. M. Brüggemann-Rotgans i Peter Diepenhorst*

Usjevi krumpira mogu žestoko biti oštećeni krumpirovim cističnim nematodama *Globodera rostochiensis* i *Globodera pallida*, koje napadaju posebno krumpir i neke druge biljke vrste *Solanaceae*. Na razvoj ličinki utječu agensi što ih izlučuje korijenje nekih vrsta *Solanaceae*. Više od sedamdeset godina mnoge skupine istraživača nisu štedjele napora kako bi izolirale i odredile strukturu tih agensa. Nažalost, svi dosadašnji pokušaji su se izjalovili. U ovom se radu opisuje određivanje strukture agensa što ga izlučuje korijenje krumpira i rajčice, a koji utječe na razvoj ličinki spomenutih nematoda. Agens razvoja pokazuje stanovitu sličnost s glicinoeklepinima kao što su ustanovili Masamune i sur. te je zbog toga nazvan solanoeklepin A.<sup>1,2-5</sup>

Kristalografski podaci:  $C_{27}H_{30}O_9 \cdot 3H_2O$ ,  $M_r = 498,5$ , monoklinski sustav, prostorna grupa  $P2_1$ ,  $a = 11,289(2)$ ,  $b = 20,644(4)$ ,  $c = 11,632(12)$  Å,  $\beta = 90,81(4)^\circ$ ,  $V = 2711(3)$  Å<sup>3</sup>,  $Z = 4$ ,  $D_x = 1,35$  g cm<sup>-3</sup>,  $\lambda(\text{Cu-K}\alpha) = 1,5418$  Å,  $\mu(\text{Cu-K}\alpha) = 9,0$  cm<sup>-1</sup>,  $F(000) = 1176$ ,  $T = -60$  °C. Konačni faktor nepouzdanosti  $R = 0.117$  za 3721 opaženih refleksa.