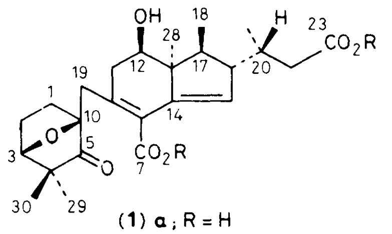
Glycinoeclepin A, a Natural Hatching Stimulus for the Soybean Cyst Nematode

Akio Fukuzawa, Akio Furusaki, Mitsuhiko Ikura, and Tadashi Masamune*
Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060, Japan

A large-scale of isolation of glycinoeclepin A, a natural hatching stimulus for the soybean cyst nematode, and its structure elucidation by spectroscopic and *X*-ray methods are described.

We recently reported[1] the isolation of the title compound, glycinoeclepin A (**1a**), as its bis(*p*-bromophenacyl) ester (**1b**). We now describe the determination of the structure of this hatching stimulus.

Compound (**1b**) was isolated in large quantities, required for the structure elucidation, as follows. Hatch-stimulating concentrates (*ca.* 1 kg) were prepared from dried and powdered roots of kidney beans (*ca.* 1 ton) collected in late July at Memuro (10 hectare), Hokkaido, and were fractionated according to an improved modification of the reported



(**1**) **a**; R = H
b; R = $CH_2COC_6H_4Br$-*p*

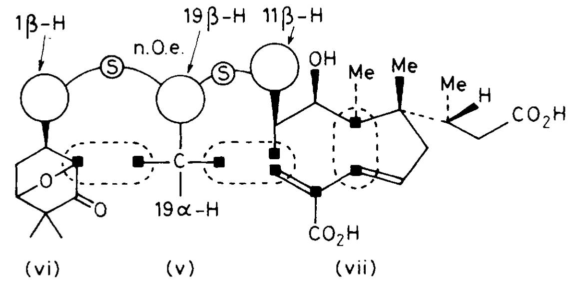


Table 1. Isolation of glycinoeclepin A as its bis(*p*-bromophenacyl) ester.

Dried and powdered kidney bean roots	1058 kg (10 ha)	
↓ Extraction with water (<10 °C, 15 kl) Evaporation (<30 °C) under reduced pressure		
Concentrates	380 l	
↓ Continuous extraction with chloroform at pH 2—3		
Chloroform extracts A	1011 g	10^{-5} g/ml
↓ EtOAc–aq. $NaHCO_3$		
Acidic extracts B	636 g	10^{-5}—10^{-6} g/ml
↓ Charcoal–Celite column (aq. acetone)		
Fraction C	197.3 g	10^{-6} g/ml
↓ Sephadex LH-20 column (MeOH)		
Fraction D	88.9 g	10^{-6}—10^{-7} g/ml
↓ DEAP-Sephadex LH-20 column (72% EtOH–AcOH–NH_4OH)		
Fraction E	18.15 g	10^{-7} g/ml
↓ Sephadex LH-20 column (CH_2Cl_2–MeOH, 95 : 5)		
Fraction F	3.59 g	10^{-7}—10^{-8} g/ml
↓ Sephadex LH-20 column (CH_2Cl_2–MeOH, 97 : 3)		
Fraction G	951 mg	10^{-8} g/ml
↓ *p*-Bromophenacylation (*p*-$BrC_6H_4COCH_2Br$, Pr^i_2NEt in MeCN, 20 °C); Silica gel column		
Fraction H as *p*-BPE[a]	2057 mg	10^{-8} g/ml
↓ Hitachi Gel 3019 column (MeOH–CH_2Cl_2, 8 : 2)		
Fraction I as *p*-BPE	336 mg	10^{-9} g/ml
↓ μBondapak C_{18} (MeOH–H_2O, 8 : 2)		
Fraction J as *p*-BPE	79 mg	10^{-10} g/ml
↓ μBondapak NH_2 (C_6H_{14}–CH_2Cl_2–MeCN, 76 : 14 : 10)		
Fraction K as *p*-BPE	8.8 mg	10^{-11} g/ml
↓ μBondapak NH_2 (C_6H_{14}–CH_2Cl_2–MeCN, 63 : 33 : 4)		
Glycinoeclepin A as *p*-BPE	1.25 mg	10^{-11}–10^{-12} g/ml

[a] *p*-BPE = bis(*p*-bromophenacyl) ester.

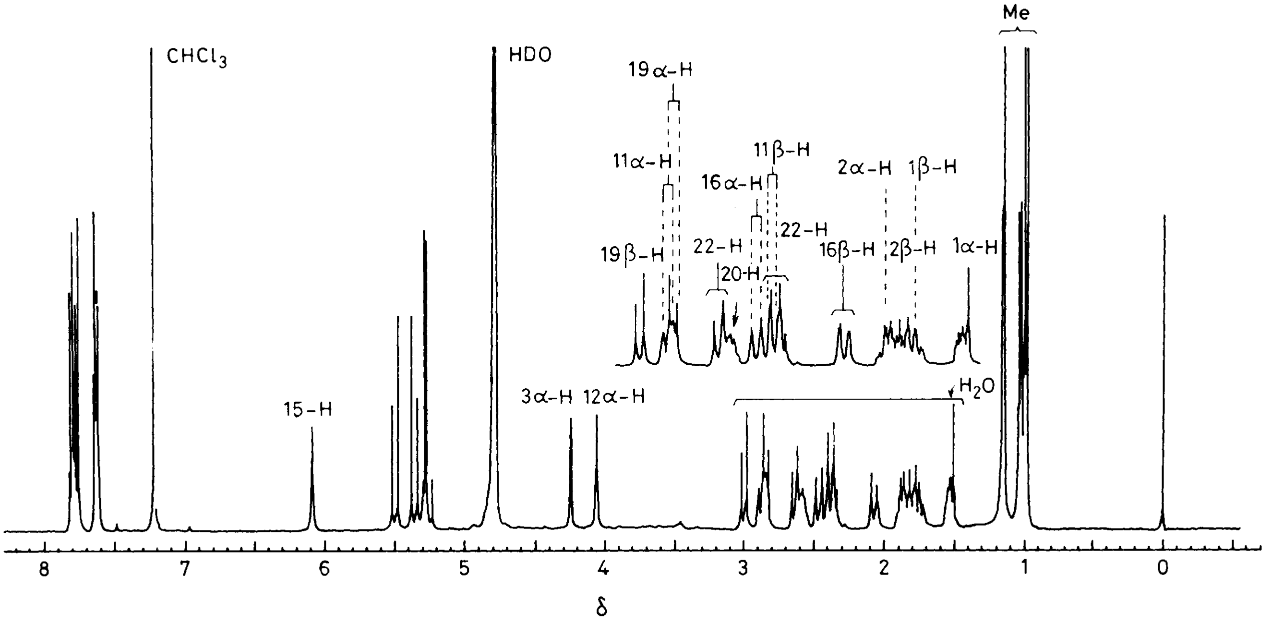


Figure 1. ¹H N.m.r. spectrum of (**1b**); 400 MHz, 500 μg in $CDCl_3$–D_2O.

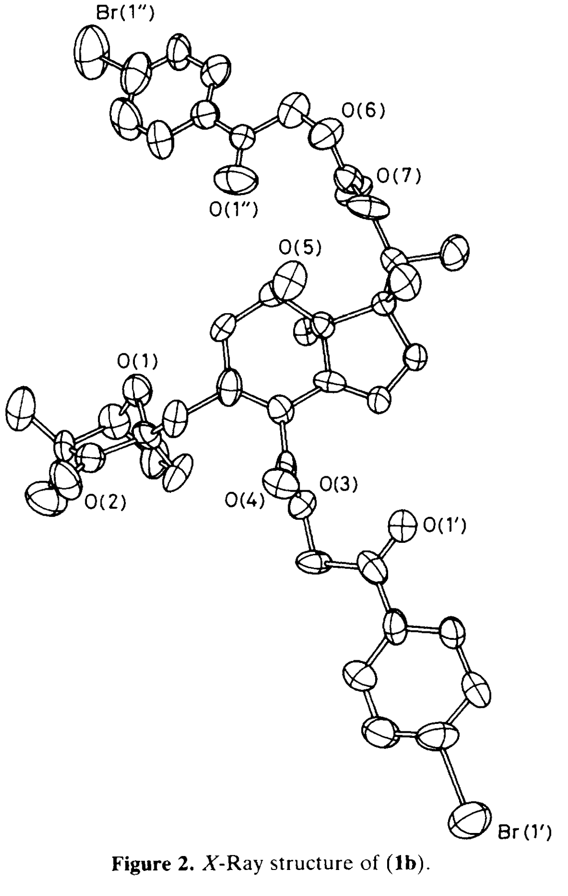


Figure 2. *X*-Ray structure of (**1b**).

procedure[1] (Table 1). Repeated chromatography, guided by bioassays,[1] yielded (**1b**) (1.25 mg), showing a single h.p.l.c. peak on various columns; following hydrolysis to give (**1a**), hatching and larval emergence were stimulated at a concentration of 10^{-11}— 10^{-12} g/ml in water at 25 °C.

The molecular formula of (**1a**) was established as $C_{25}H_{34}O_7$ on the basis of the field-desorption (*m/z* 838) and chemical-ionisation (NH_3; *m/z* 856) mass spectra of (**1b**) as well as the ¹H n.m.r. spectra of (**1b**) (Figure 1). The ¹³C n.m.r. spectra of (**1b**) under completely decoupled and off-resonance conditions, combined with INEPT studies, and the ¹H n.m.r. spectra of (**1b**), coupled with examination of the COSY spectra and extensive decoupling studies, indicated the presence of the following structural units:† 2 × (CH_3-■), CH_3-■-CH_3, >C=O (δ 215.7), sp^3-C-CO_2H (δ 173.1), ■=■-CO_2H (δ 169.5), ■-CH(O-■)CH_2CH_2-■ (i), ■-CH(CH_3)CH_2-■ (ii), ■-CH(OH)CH_2-■ (iii), ■=C*H*(δ 6.02)CH_2-■ (iv), and ■-CH_2-■ (v). Measurement of the nuclear Overhauser effect (n.O.e.) difference spectra of (**1b**) involving the methyl and other protons, coupled with the i.r. (1758 cm^{-1}) and u.v. [251 nm (ε 8300)] spectra of (**1a**), led to the presumption that the unit (i) can be extended to a structural moiety (vi) and units (ii)—(iv) to the unit (vii). In view of the small n.O.e. between 19β-H in (v) and 1β-H (vi) and 11β-H in (vii), the partial structures (vi), (v), and (vii) must be connected straightforwardly, and hence glycinoeclepin A most probably has the structure (**1a**).

This structure assignment was confirmed, and the absolute configuration established, by *X*-ray analysis of a single crystal of (**1b**) obtained by slow evaporation of a solution in hexane and dichloromethane containing a trace of methanol. *Crystal data:* $C_{41}H_{44}O_9 \cdot \frac{1}{2}CH_2Cl_2$, monoclinic, space group $P2_1$, a = 18.596(6), b = 15.010(4), c = 16.112(5) Å, β = 114.41(2)°, Z = 4, D_c = 1.432 g cm^{-3}, μ(Cu-K_α) = 35.6 cm^{-1}. There are two molecules of (**1b**) and one of dichloromethane in the asymmetric unit. The structure was solved by the Monte Carlo direct method,[2] and the absolute configuration was determined by taking account of the anomalous dispersion of the halogen atoms for Cu-K_α radiation. The final R value was 7.8%. The two molecules of (**1b**) have almost the same

---

† ■ denotes a quaternary carbon atom.

geometry, and the structure of one is shown in Figure 2.‡ The structure of (**1a**) is characterized by migration of two methyl groups involved in the C and D rings and oxidative cleavage of the B ring with loss of one carbon atom, compared with those of cycloartanes such as cimigenol,[3] and is regarded as a pentanortriterpene.

Received, 2nd July 1984; Com. 945

References

1 T. Masamune, M. Anetai, M. Takasugi, and N. Katsui, *Nature (London)*, 1982, **297**, 494.
2 A. Furusaki, *Acta Crystallogr., Sect. A*, 1979, **35**, 220.
3 G. Kusano, H. Uchida, Y. Murakami, N. Sakurai, and T. Takemoto, *Yakugaku Zasshi*, 1976, **96**, 321.

---

‡ The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.