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GLYCINOECLEPINS B AND C, NORTRITERPENES RELATED TO GLYCINOECLEPIN A

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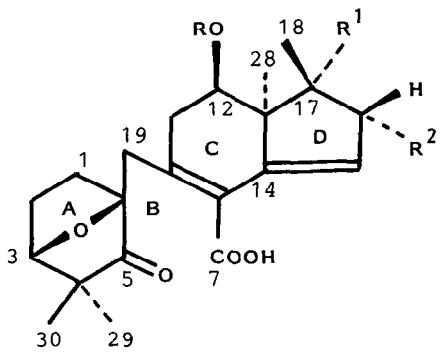
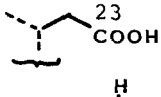
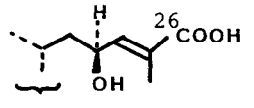
Summary: The structure of two new nortriterpenes, glycinoeclepins B and C, isolated from the aqueous extracts of roots of kidney bean and related structurally to glycinoeclepin A, a natural hatching stimulus for the soybean cyst nematode, has been elucidated to be formulas 2 and 3, respectively.

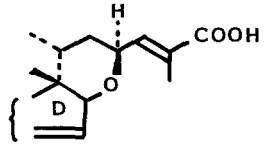
We recently reported the isolation[1,2] and structure[2] of a natural hatching stimulus, glycinoeclepin A (GEA) (1), for the soybean cyst nematode. In a continuing study aimed at search of the active principles from dried and powdered roots of kidney bean (Phaseolus vulgaris L.), we have isolated two new nortriterpenes, designated as glycinoeclepins B (GEB) (2) and C (GEC) (3). The isolation and structure elucidation are described in this communication.

The fraction J[1,2] [79 mg as its bis(p-bromophenacyl) ester (p-BPE)], which had been obtained by fractionation of the hatch-stimulating concentrates (≈1 kg), had been separated into 7 fractions by preparative HPLC over μBondapak NH_2 with hexane:dichloromethane:acetonitrile (76:14:10). One (fraction 4) (4.9 mg) of the three major fractions was subjected to preparative HPLC over the same column with the solvent mixture (60:36:4) to yield GEC p-BPE (3a) (1.8 mg), which on hydrolysis (0.5M KOH in MeOH, 45 °C, 16 h) gave GEC (3). On the other



1 (GEA) R=H, R^1=  , R^2=H

2 (GEB) R=Ac, R^1=  , R^2=H

3 (GEC) R=H, R^1,R^2= 

hand, fraction 5 (8.8 mg) (= fraction K[1,2]) was separated into two fractions under almost the same conditions [μBondapak NH_2, the solvent mixture (63:33:4)]. One fraction gave GEA p-BPE (1a) (1.25 mg),[2] while another was again purified by preparative HPLC over μBondapak NH_2 with the solvent mixture (63:13.2:8.8) to give GEB p-BPE (2a) (1.8 mg). The ester (2a) underwent smooth hydrolysis to yield GEB (2), which was converted into deacetylglycinoeclepin B (2b) by treatment under more severe conditions (5% NaOMe in MeOH, 70 °C, 11 h).

The molecular formula of GEB (2) was established as $C_{31}H_{42}O_9$ on the basis of the FD mass spectrum [m/z 950, 952, and 954 (1:2:1)] of 2a as well as the ^1H NMR spectrum[3] (Fig. 1) of 2a. The ^{13}C NMR spectrum[3] of 2a under completely decoupled and off-resonance conditions, combined with INEPT studies, and the ^1H NMR spectrum of 2a, coupled with examination of the COSY spectra and extensive decoupling studies, indicated the presence of the following structural units: (i) ■-CH(O-■)CH_2CH_2-■, CH_3-■-CH_3, and C(δ 216.7)=O (1750 cm^{-1});[3] (ii) ■-CH_2-■; (iii) ■-CH(OAc)CH_2-■, 2x(CH_3-■), and ■=■[C(δ 167.7)OOH]-■=CH(δ 6.02)CH_2-■ [2, UV_{max} 252 nm (ε 9500)];[3] (iv) ■-CH(CH_3)CH_2CH(OH)CH(δ 6.89)=C(CH_3)-■ and C(δ 167.7)OOH.[4] These units (i)-(iv) are corresponding to the A, B (cleaved), C and D rings, and the side chain, respectively, since the result of measurement of the NOE difference spectra and of estimation of the coupling constants between the protons involved in the units (i)-(iii) (Fig. 2) was essentially the same as that in GEA p-BPE[2] (1a). In view of the coocurrence of GEA and GEB, GEB evidently possesses the same skeleton as GEA, deffering from GEA only in the substituent (OH → OAc) at C-12 and the side chain. The structure of the side chain [the unit (iv)] was elucidated as shown in Fig. 3 on the basis of the NOE and the coupling constants (Hz) between the protons in question [$J_{20,22\beta}$ = 0, $J_{20,22\alpha}$ = 11.1, J_{22gem} = 12.8 (cf., 1a, $J_{20,22}$ = 0 and 11.5, J_{22gem} = 14.5), $J_{22\beta,23}$ = 12.8, $J_{22\alpha,23}$ = 2.0, $J_{23,24}$ = 8.2, and $J_{24,27}$ = 1.2], indicating the (23S) and (24E) configuration. GEB is therefore represented most favorably by formula 2.

GEC (3) had molecular formula $C_{29}H_{38}O_9$ [FD-MS of 3a, m/z 906, 908, and 910 (1:2:1)] and consisted of the following structural units: (i) ■-CH(O-■)CH_2CH_2-■, CH_3-■-CH_3, and C(δ 215.9)=O (1754 cm^{-1}); (ii) ■-CH_2-■; (iii) ■-CH(OH)CH_2-■, 2x(CH_3-■), and ■=■[C(δ 166.8 or 167.2)OOH]-■=CH(δ 6.22)CH(O-)-■ [3, UV_{max} 253 nm (ε 9500)]; (iv) ■-CH(CH_3)CH_2CH(O-)CH(δ 6.88)=C(CH_3)-■, and C(δ 167.2 or 166.8)OOH. These units were revealed on the basis of the spectral data, specially of the ^1H and ^{13}C NMR spectra of 3a. Detailed examination of the NOE difference spectra between methyl and other protons involved in the units (i)-(iii) indicated that GEC possessed the same skeleton as GEA with C-16 substituted by an ether oxygen, involving the units (i)-(iii) corresponding to the A, B (cleaved), and C and D rings. The (16R,23S,24E) configuration in the D ring and the side chain was deduced from the spectral ground shown in Fig. 4. GEC is therefore represented best by formula 3.

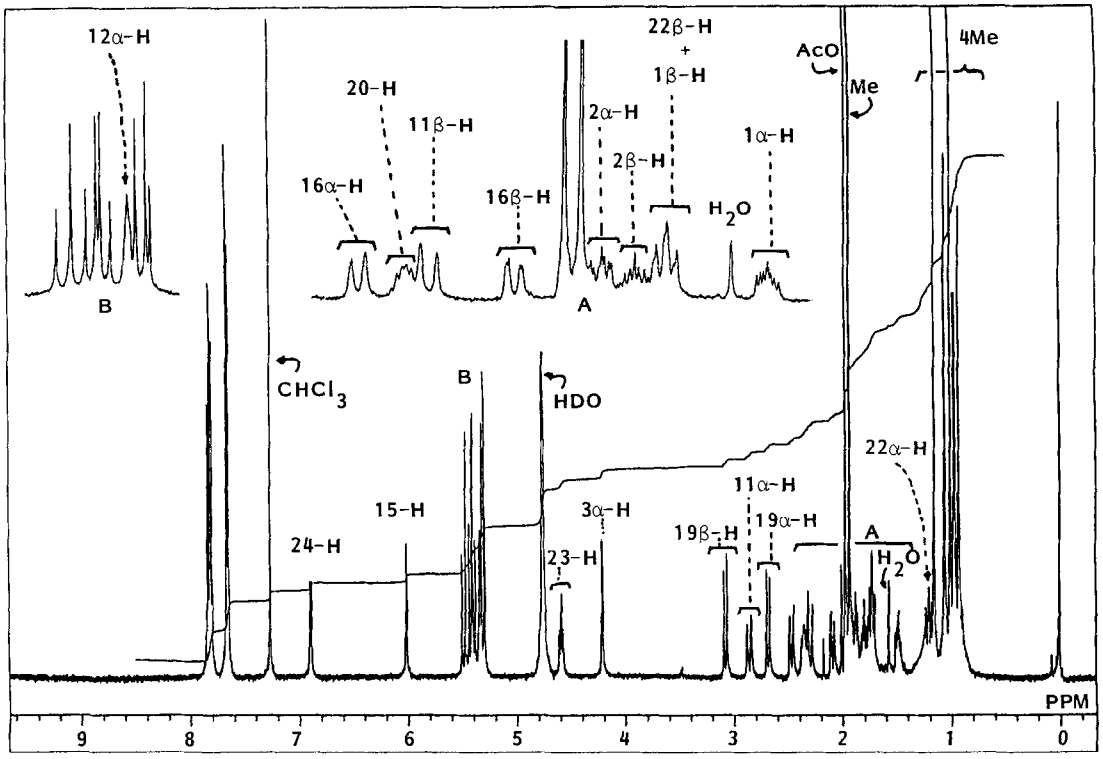


Figure 1. The ^{1}H NMR spectrum of GEB p-BPE (2a); 500 MHz, 1 mg in $CDCl_3$-D_2O

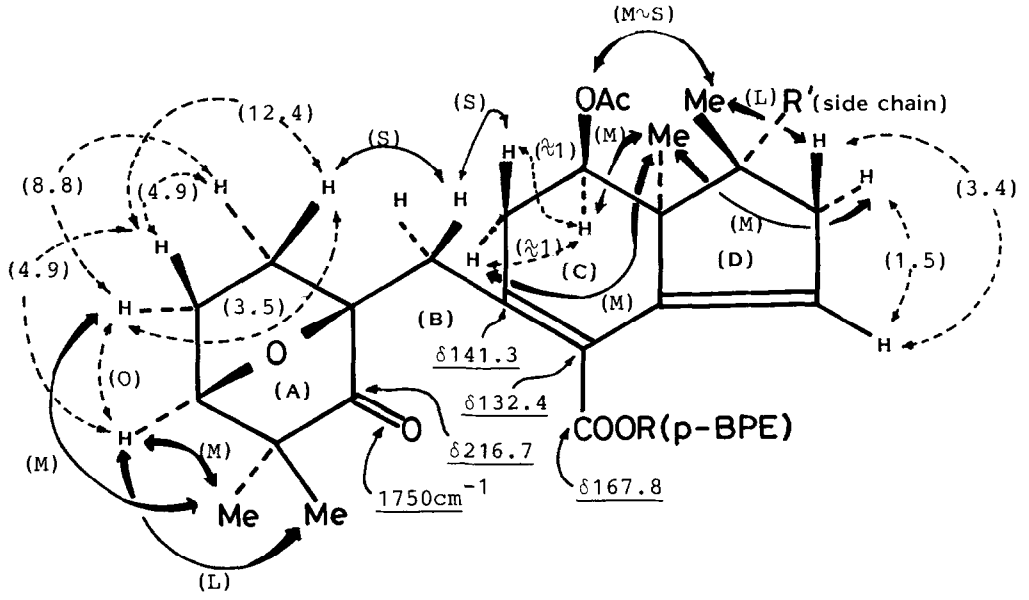


Figure 2. The NOE (↶↷) and coupling constants (Hz) between the protons in the A→D ring of GEB p-BPE (2a): (S, small; M, medium, L, large)

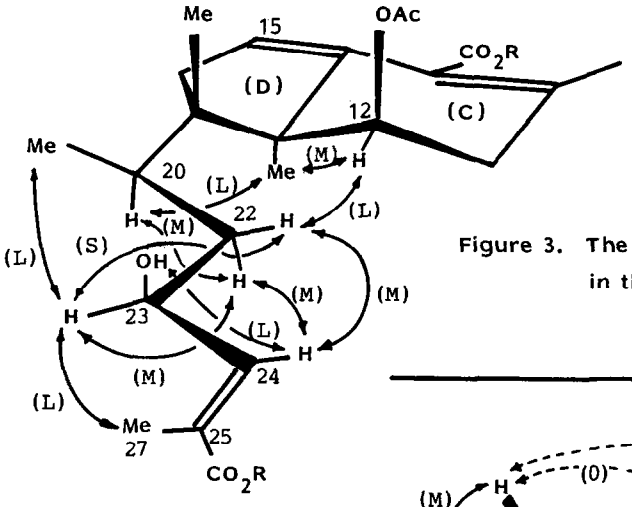


Figure 3. The NOE (↷) between the protons in the side chain of GEB p-BPE (2a)

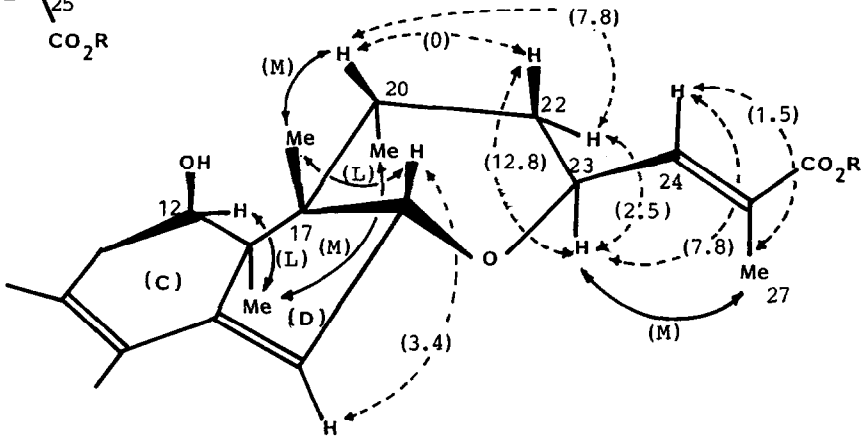


Figure 4. The NOE (↷) and coupling constant (Hz) between the protons in the side chain of GEC p-BPE (3a)

Deacetylglycinoeclepin B (2b) stimulates the hatching of the soybean cyst nematode eggs at $10^{-8\sim-9}$ g/ml [cf., GEA (1), active at $10^{-11\sim-12}$ g/ml] in water at 25 °C, but GEB (2) and GEC (3) show no activity for the hatching at 10^{-7} g/ml.

References and Notes

1) T. Masamune, M. Anetai, M. Takasugi, and N. Katsui, Nature (London), (1982). 297, 495.

2) A. Fukuzawa, A. Furusaki, M. Ikura, and T. Masamune, J. Chem. Soc., Chem. Commun., (1985) 222.

3) The ^{1}H and ^{13}C NMR spectra and the IR and UV spectra were measured in $CDCl_3$ (or C_6D_6) (500 or 400 MHz), C_6D_6 (25 or 125 MHz), KBr, and MeOH, respectively.

4) The mark ■ denotes a quaternary carbon atom.

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