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Glycinoeclepins, Natural Hatching Stimuli for the Soybean Cyst Nematode, *Heterodera Glycines.* II. Structural Elucidation

Tadashi Masamune,* Akio Fukuzawa, Akio Furusaki, Mitsuhiko Ikura, Hideki Matsue,
Tatsuya Kaneko, Atsushi Abiko, Noriyasu Sakamoto,
Norihiko Tanimoto, and Akio Murai
Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060
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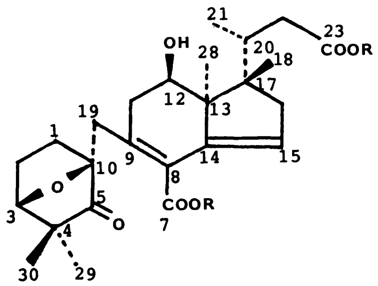
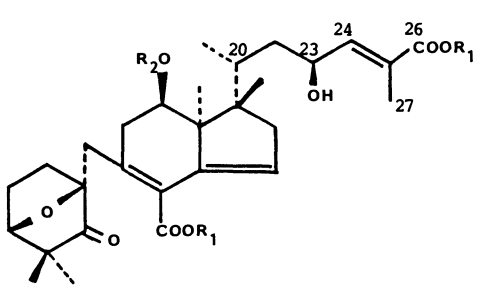
The structures of three new compounds, glycinoeclepins A, B, and C, isolated from the hatch-stimulating extracts of kidney beans for the soybean cyst nematode, have been determined. The structures were resolved by using spectroscopic methods, especially by the difference NOE techniques, and confirmed by the X-ray diffraction analysis. These structures are characterized by (1) migration of two methyl groups in the C and D rings, (2) oxidative cleavage of the B ring with loss of one carbon atom and (3) presence of the 7-oxabicyclo[2.2.1]heptane system in the A ring, compared with those of cycloartanes, and are regarded as pentanor- (GEA) or nortriterpenes (GEB and GEC).

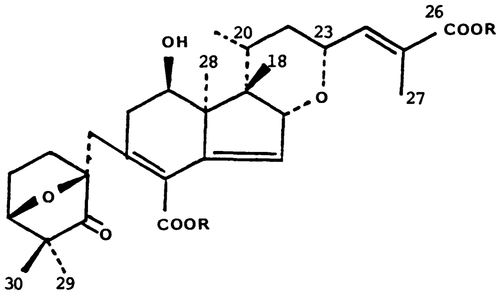
In the preceding paper[1] we described the isolation of natural hatching stimuli, designated as glycinoeclepins A, B, and C (GEA, GEB, and GEC), for the soybean cyst nematode as their *p*-bromophenacyl esters (p-BPEs). Of these compounds, GEA, identified as an active principle, stimulated the hatching at 10^{-11}—$^{-12}$ g ml^{-1} in water at 25 °C.[2] In preliminary communications,[3,4] we reported the structures of these stimuli. However, it has recently been found that the reported (20*R*, 23*S*, 24*E*) configuration[4,5] of the side chain of GEC should be revised to the (20*R*, 23*R*, 24*E*) one. The present paper describes the details of the structural elucidation of GEs including the structural revision of GEC.



GEA (**1**), R=H
GEA p-BPE (**1a**), R=$CH_2COC_6H_4Br$



GEB (**2**), R_1=H, R_2=$COCH_3$
GEB p-BPE (**2a**), R_1=$CH_2COC_6H_4Br$, R_2=$COCH_3$
Deacetyl GEB (**2b**), R_1=H, R_2=H



GEC (**3**), R=H
GEC p-BPE (**3a**), R=$CH_2COC_6H_4Br$

Structural Elucidation of GEA on the Basis of the Spectroscopic Evidence. The molecular formula of GEA (**1**) was determined as $C_{25}H_{34}O_7$ on the basis of the FD-MS [*m/z* 838, 840, and 842 (1:2:1)] and $CI(NH_3)$-MS spectra [*m/z* 856, 858, and 860 (1:2:1)] of GEA p-BPE (**1a**) as well as the ^1H NMR spectra of **1a** [$CDCl_3$ (Fig. 1) and C_6D_6]. The ^{13}C NMR spectra of **1a** ($CDCl_3$ and C_6D_6) under completely decoupled (Table 1) and off-resonance conditions, combined with the INEPT studies, indicated the presence of 5 methyl, 6 methylene, 1 methine, and 3 quaternary (■) carbon atoms, 2 methine and 1 quaternary carbon atoms bearing oxygen atoms, 1 trisubstituted and 1 tetrasubstituted double bonds, and 2 carboxyl and 1 carbonyl carbon atoms. These spectral data, coupled with IR spectrum of **1a** (1758 cm^{-1}, sh), revealed the presence of the following oxygen functions: 2 -COOH, 1 -OH, 1 -O-, and 1 $-\overset{|}{C}=O$ (ketone). Moreover, the ^1H NMR spectra of **1a**, combined with the detailed analysis of the COSY spectra (Fig. 2) and extensive decoupling studies, indicated the presence of structural units (i) to (x) as shown in Table 2. However, confirmatory evidence was required for the presence of the units (iii) and (iv), because the coupling constants between vicinal protons in the units [unit (iii), ■-C(12)H(OH)-$C(11)H_2$-■, $J_{12\alpha,11\beta}$=

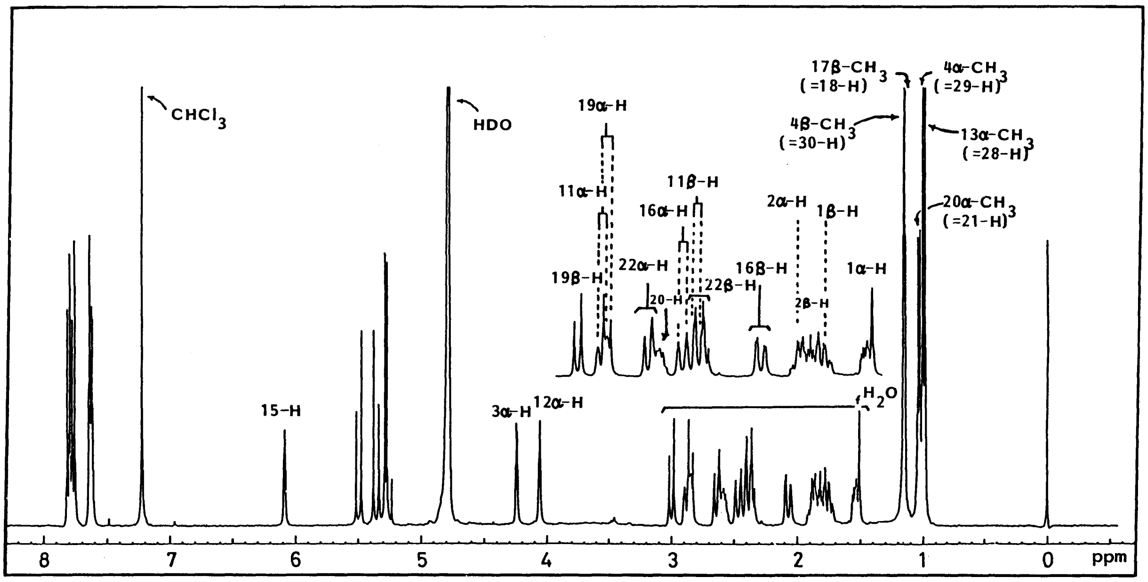


Fig. 1. The ^{1}H NMR spectrum of GEA p-BPE (**1a**) (400 MHz, 0.5 mg in $CDCl_3$ and D_2O).

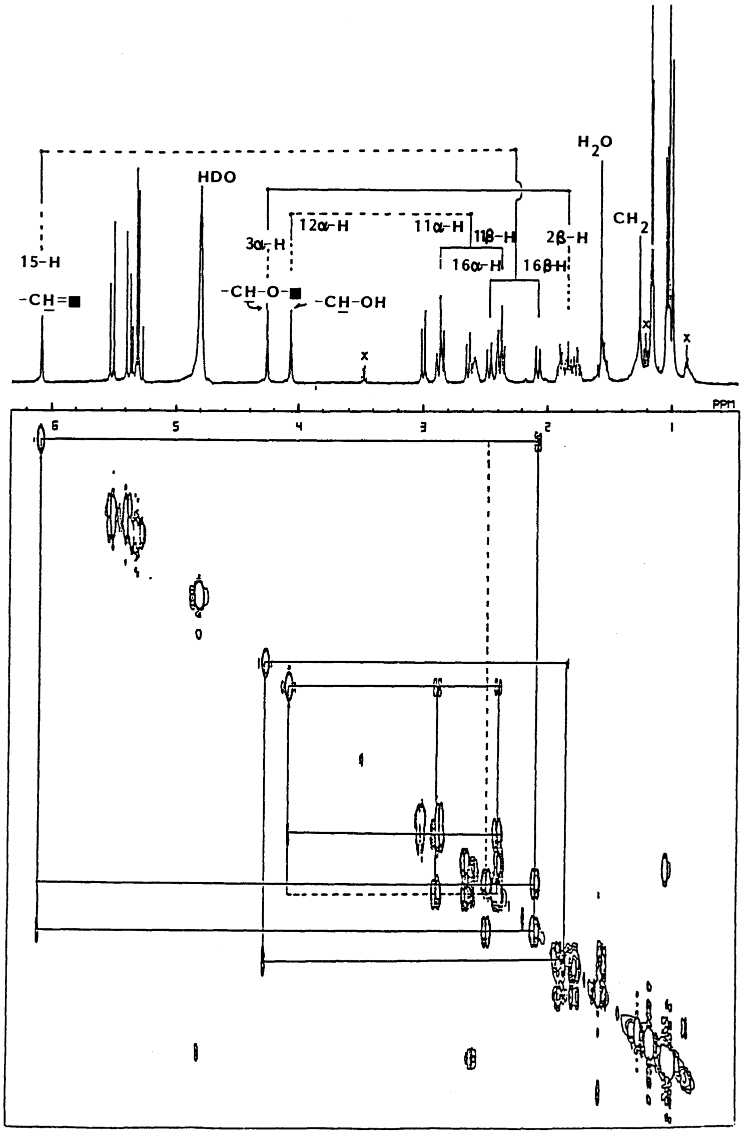


Fig. 2. The COSY spectrum of GEA p-BPE (**1a**) (500 MHz, 1 mg in $CDCl_3$ and D_2O).

1.5 Hz and $J_{12\alpha,11\alpha}$=3 Hz; unit (iv), ■=C(15)H-C(16)H$_2$-■, $J_{15,16\beta}$=3.5 Hz, $J_{15,16\alpha}$=1.5 Hz] were less than 4 Hz and hence might be attributed to long-range coupling. Thus the NOE difference spectra of these vicinal protons were measured. The result clearly demonstrated that the protons in question in the respective units are situated in a narrow region each other, and the C(11) and C(15) atoms are connected directly to the C(12) and C(16) atoms, respectively.

The following comments are annotated on Tables 1 and 2. (1) The olefinic proton H$_{15}$ in the unit(iv) of **1a** was observed at δ 6.08 (cf., **2a**, δ 6.02; **3a**, δ 6.22), at rather low-field compared with usual olefinic protons. (2) Two *p*-bromophenacyloxycarbonyl carbon atoms in the units(viii) and (ix) of **1a** appeared with clearly different chemical shifts (δ 167.6 and 173.1, Δδ 5—6 ppm) (Table 1), while the corresponding atoms in **2a** and **3a** did at practically the same field (**2a**, δ 167.8 and 167.8; **3a**, δ 167.2 and 167.6) (Table 1). These facts are consistent with that one of the relevant carbon atoms is connected to an sp^3-type carbon atom [unit(ix)] and another to an sp^2-type carbon atom [unit(viii)] in **1**. (3) The chemical shift (δ 216.6) of one carbonyl carbon atom [unit(x)] of **1a** strongly suggested that the carbonyl group would be sterically hindered and/or involved in a strained system (cf., **2a**, δ 216.7; **3a**, 216.3).[6] All these facts will be discussed again.

Table 1. Chemical Shift of the ^{13}C Atoms of GEA (**1**), GEB (**2**), and GEC (**3**) in the ^{13}C NMR Spectra of their p-BPEs

	1 (22.5 and 25 MHz)		**2**(25 MHz)	**3**(125 MHz)
Solvent	CDCl$_3$	C$_6$D$_6$	C$_6$D$_6$	C$_6$D$_6$
*C*H$_3$-	16.2	17.0	16.4	16.8
	18.4	19.3	18.9	20.3
	19.1	19.8	19.7	23.3
	19.8	20.3	20.4	24.8
	22.7	23.3	23.5	29.2
			13.4$^{a)}$	13.8$^{a)}$
			22.0$^{b)}$	
-*C*H$_2$-	25.1	25.8	25.9	25.8
	29.4	29.8	29.5	30.1
	33.5	34.4	34.3	34.8
	38.0	38.9	39.2	36.8
	40.9	42.2	40.8	42.0
	46.0	46.9	47.5	
$-\overset{\vert}{C}H-$	35.1	36.0	34.3	40.4
$-\overset{\vert}{\underset{\vert}{C}}-$ (sp^3)	48.7	49.1	49.3	47.5
	51.2	52.2	52.3	49.2
	53.3	54.1	52.3	54.6
-*C*H(OH)-	71.3(d)	71.9(d)	67.4(d)$^{c)}$	73.3(d)
-*C*H(O-)-	83.5(d)	84.0(d)	73.3(d)	75.4(d)
$-\overset{\vert}{C}(O-)-$	88.4(s)	89.3(s)	84.2(d)	84.1(d)
			89.3(s)	84.1(d)
				91.0(s)
$-\overset{\vert}{C}=CH-$	126.4(d)	126.7(d)	125.5(d)	126.1(d)
$-\overset{\vert}{C}=\overset{\vert}{C}-$	127.2(s)	126.7(s)	128.8(s)	132.4(d)
	133.6(s)	134.4(s)	129.1(s)	137.9(s)
	138.9(s)	140.8(s)	134.6(s)	144.5(s)
			141.5(s)	144.5(s)
			146.8(d)	147.2(s)
-*C*OO-p-BPE	166.8	167.6	167.8	167.2
	172.7	173.1	167.8	167.6
-*C*(=O)-	216.9	216.6	216.7	216.3

a) -CH(*C*H$_3$)=CH-. b) -OC(=O)*C*H$_3$.
c) -*C*H(OCOCH$_3$)-.

Now we must connect these structural units(i) to (x), which are disconnected by ten quaternary (■) carbon atoms. The NOE difference spectra[7,8] (Fig. 3) between protons of two geminal methyl groups [unit(vii)] and those in the unit(i) indicated that (1) one [■=C(4)] of the terminal quaternary carbon atoms in the unit(i) must be identical with that of the unit(vii), and (2) another terminal quaternary carbon [■=C(10)] of the unit(i) must be identical with that bearing the ether oxygen atom, because (a) neither of the two protons (H$_{1\beta}$ and H$_{1\alpha}$) at the C(1), attached to C(10), is located close to all other protons involved in the units(ii) to (vi), and (b) if the relevant carbon C(10) were identical with the former C(4), forming a four-membered ring, the coupling constants between geminal protons at C(1) and C(2) would be much smaller than those observed ($J_{1\beta,1\alpha}=J_{2\beta,2\alpha}$=12 Hz). Hence the units(i) and (vii) can be extended to a structural moiety (A), in which the coupling constants between protons at C(3) and C(2) were $J_{3\alpha,2\beta}\doteqdot$ 5 Hz and $J_{3\alpha,2\alpha}\doteqdot$0 Hz. These constants led us to presume that the moiety (A) must be involved in a 7-oxabicyclo[2.2.1]heptane system. This skeletal system indeed exists as the A ring in acerinol[9] (**4**), a triterpene of natural origin. The coupling con-

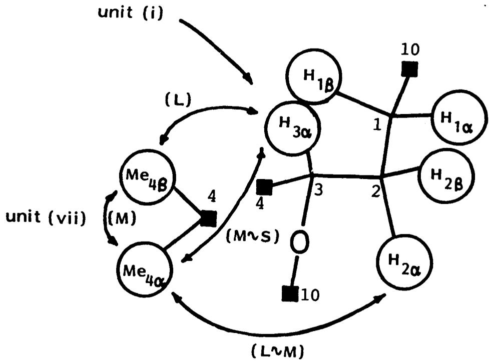
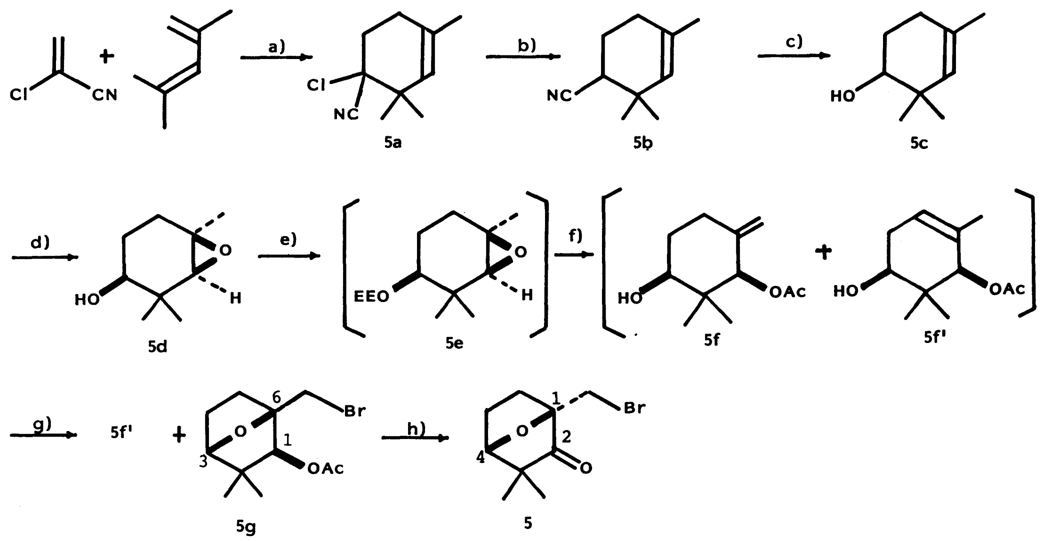


Fig. 3. The difference NOEs (↶↷) between the geminal methyl and other protons in GEA p-BPE (**1a**).

stants between protons in the A ring of **4** were readily obtained by measurement of the decoupling difference spectrum (Fig. 4), the result being tabulated in Table 3. Table 3 revealed that these coupling constants were essentially the same as those of the protons in the moiety (A), indicating that the moiety (A) can be extended to a 4,4-dimethyloxabicyclo[2.2.1]heptane moiety[10] (B) with an sp^2-type carbon atom at C-5 (a carbonyl carbon or an olefinic carbon). Then we prepared 1-bromomethyl-3,3-dimethyl-7-oxabicyclo[2.2.1]heptan-2-one (**5**) (Scheme 1) and compared the spectral data with those of **1a**



Scheme 1. Preparation of 1-bromomethyl-3,3-dimethyl-7-oxabicyclo[2.2.1]heptan-2-one (**5**). Reagents: a) Δ (80%), b) Zn-Cu (98%), c) LDA, O_2, $P(OEt)_3$; $NaBH_4$ (62%), d) *t*-BuOOH, $VO(acac)_2$ (95%), e) $EtOCH=CH_2$, PPTS (94%), f) $LiNEt_2$, HMPA; Ac_2O, Py; HCl (35%), g) NBS (33% and 33%), h) K_2CO_3; CrO_3, H_2SO_4 (99%).

Table 2. Structural Units of GEA (**1**), GEB (**2**), and GEC (**3**)

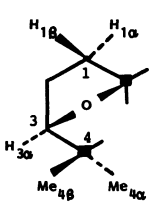
Unit	GEA ($C_{25}H_{34}O_7$)	GEB ($C_{31}H_{42}O_9$)	GEC ($C_{29}H_{38}O_8$)
(i)	■-CH(O-■)CH_2CH_2-■	■-CH(O-■)CH_2CH_2-■	■-CH(O-■)CH_2CH_2-■
(ii)	■-CH(CH_3)CH_2-■	■-CH(CH_3)CH_2CH(OH)CH=C(CH_3)-■	■-CH(CH_3)CH_2CH(O-)CH=C(CH_3)-■
(iii)	■-CH(OH)CH_2-■	■-CH($OCOCH_3$)CH_2-■	■-CH(OH)CH_2-■
(iv)	■=$CHCH_2$-■	■=$CHCH_2$-■	■=CHCH(O-)-■
(v)	■-CH_2-■	■-CH_2-■	■-CH_2-■
(vi)	CH_3-■, CH_3-■	CH_3-■, CH_3-■	CH_3-■, CH_3-■
(vii)	CH_3-■-CH_3	CH_3-■-CH_3	CH_3-■-CH_3
(viii)	■=■-■OOH	■=■-■OOH	■=■-■OOH
(ix)	(C_{sp^3})-■OOH	(C_{sp^2})-■OOH	(C_{sp^2})-■OOH
(x)	■=O	■=O	■=O

Table 3. The Chemical Shifts (δ) and Coupling Constants (*J*) of the Protons in the Structure Moiety (A) of GEA p-BPE (**1a**) and the A ring of Acerinol (**4**)[a]

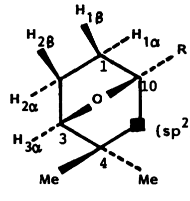
Protons	GEA p-BPE (moiety A)		Acerinol (A ring)	
	δ	*J* (Hz)[b]	δ	*J* (Hz)[b]
1α-H	1.54	(ddd, *J*=4, 9, 12)	1.60	(ddd, *J*=4.5, 9.5, 11.5)
1β-H	1.76	(ddd, *J*=5, 10, 12)	1.41	(ddd, *J*=5, 11.5, 11.5)
2α-H	1.90	(ddd, *J*=5, 9, 12)	1.93	(ddd, *J*=5, 9, 12.5)
2β-H	1.84	(dddd, *J*=4, 5, 10, 12)	1.71	(dddd, *J*=4.5, 5, 11.5, 12.5)
3α-H	4.26	(d, *J*=5)	3.73	(d, *J*=5)

a) The spectra were measured in $CDCl_3$. b) The coupling constants were estimated at the 1st order approximation.

[IR (KBr), **1a**, 1760 cm^{-1} (sh) (**1**, 1757 cm^{-1}) (C=O); **5**, 1757 cm^{-1}: ^{13}C NMR ($CDCl_3$), **1a**, δ 216.9 (C=O); **5**, δ 214.6: ^{1}H NMR ($CDCl_3$ and C_6D_6), **1a**, δ *1.02 and 0.73* (4α-CH_3), and 1.17 and 1.10 (4β-CH_3); **5**, δ *1.06 and 0.66* (3α-CH_3), and 1.23 and 1.04 (3β-CH_3)]. The absorption peaks due to the carbonyl group in the IR (near 1760 cm^{-1}) and ^{13}C NMR spectra (near δ 215 ppm), coupled with the up-field shift ($\Delta\delta \approx 0.4$ ppm) of α-oriented methyl protons in the ^{1}H NMR spectrum in hexadeuteriobenzene, have established that a 4,4-dimethyl-3,10-epoxy-5-cyclohexanone moiety[10] (C) is involved in GEA (**1**).



A



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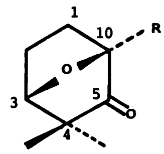
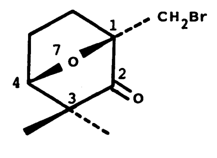


C



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We next discuss on a structural moiety constructed by the units(ii), (iii), (iv), and (vi) in Table 2. The NOE difference spectra[7,8] between the protons of these units of **1a** were measured in details, the result being summarized in Fig. 5. As shown in Fig. 5, the protons of one (17β-CH_3=18-H) of two methyl groups in the unit(vi) were located close to secondary methyl protons (20β-CH_3=21-H)[11] and one proton (22β-H)[11] in the unit(ii), a hydroxyl proton (measured in C_6D_6) in the unit(iii), and two protons (16β-H and 15-H) in the unit(iv), while another methyl protons (13α-CH_3=28-H) were situated close to most of the remaining protons of the units(ii) (20α-H and 22α-H), (iii) (12α-H and 11α-H), and (iv) (16α-H). These findings revealed that (1) three major C–C bonds of the units(ii) to (iv) constitute an almost flat plane, and (2) half (20β-CH_3, 22β-H, OH, and 16β-H) of these protons and one methyl protons (17β-CH_3) are disposed at an upper (or lower) side of the plane, and the remaining protons (20α-H, 22α-H, 12α-H, 11α-H, and 16α-H) and another methyl protons (13α-CH_3) are located at a lower (or upper) side of the plane. It is emphasized that any notable NOE was not observed between these protons of the units(ii), (iii), (iv), and (vi) and other protons of the units(i) and (vii). We had to connect these units so as to form a structural moiety consistent with all the spectral data, although a number of combinations of the units were possible.

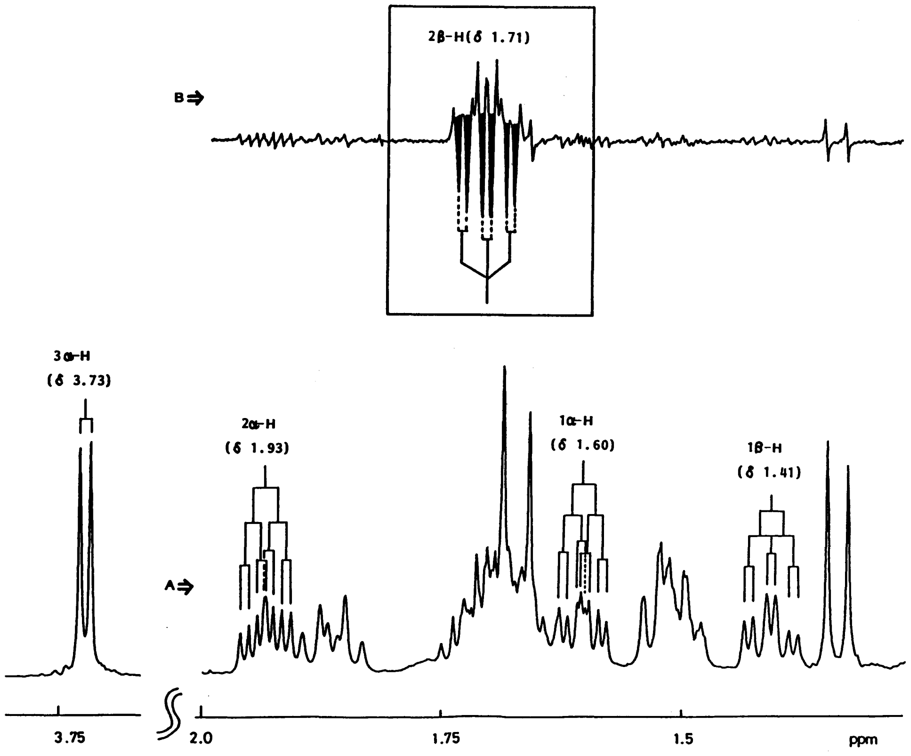
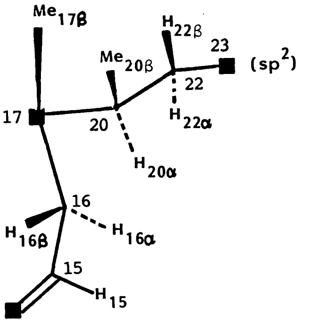
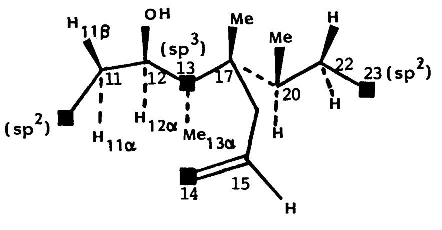


Fig. 4. The ^{1}H NMR spectrum of the A-ring of acerinol (**4**) (500 MHz, $CDCl_3$).
(A) The normal spectrum: (B) The decoupling difference spectrum on irradiation at δ 3.73 (3α-H).

As indicated in Fig. 5, the secondary methyl protons (20β-CH_3)[11] in the unit(ii) were located near to both two geminal protons (16β-H and 16α-H) in the unit(iv) and the methyl protons (17β-CH_3), attached to a quaternary carbon [C(17)] and disposed at the upper side of the plane. The latter protons (17β-CH_3) were also situated close to one proton (22β-H) in the unit(ii) and one proton (16β-H) in the unit(iv). Thus one terminal quaternary carbon atom of the unit(ii) and that of the unit(iv) must be identical with the quaternary carbon atom C(17), forming a structural moiety (D). The same discussion holds for relation between another methyl protons (13α-CH_3), attached to a quaternary carbon atom [C(13)] and located at the lower side of the plane, two protons (12α-H and 11α-H) in the unit(iii), and half (20α-H, 22α-H, and 16α-H) of the protons in the moiety (D). Hence one terminal quaternary carbon atom of the unit(iii) must be identical with that C(13), which must be connected directly to that C(17) in the moiety (D). It follows that the moiety (D) can be extended to a structural moiety (E).



D



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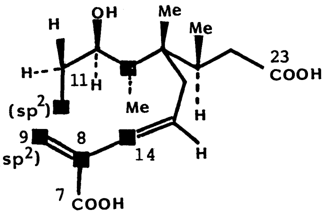


F

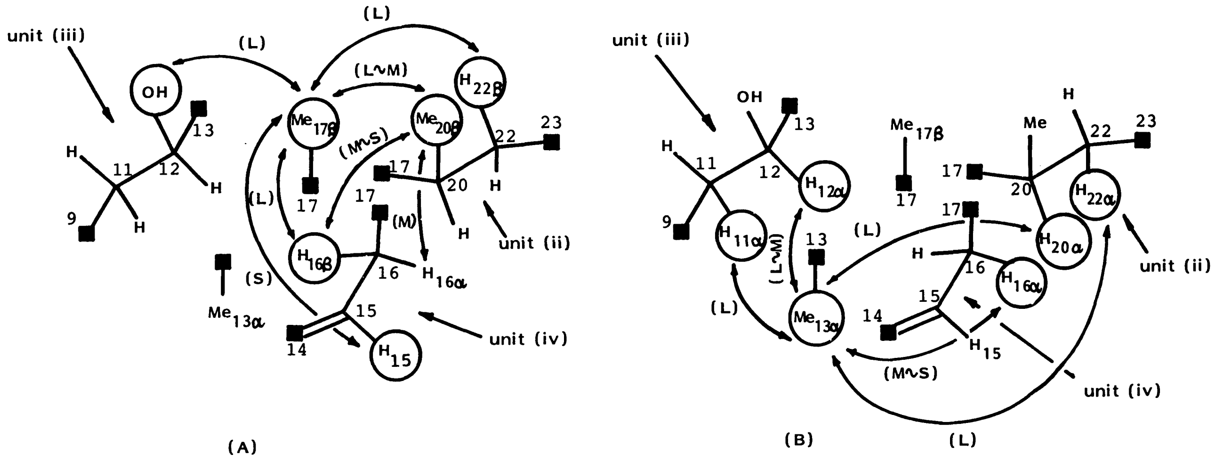


Fig. 5. The difference NOEs (↶) (A) and (B) between the two methyl (unit vi) and other protons in GEA p-BPE (**1a**).

The moiety (E) is further extended to a structural moiety (F) on the basis of the following reasons. (1) GEA (**1**) showed an absorption maximum at 251.5 nm (ε 8300) in the UV spectrum [cf., **1a**, 254.5 nm (ε 27100)] and a sharp signal (W_H=3 Hz) due to an olefinic proton at δ 5.66 in the ^{1}H NMR spectrum (CD_3OD) [cf., **1a**, δ 6.08 ($CDCl_3$) and 6.54 (C_6D_6)]. These data indicated that two double bonds in the units(iv) [and hence in the moiety (E)] and (viii) are conjugated in a cross-conjugation manner. (2) Two protons (22β-H and 22α-H) in the unit(ii) [hence in the moiety (E)] appeared at δ 2.64 and 2.37 in the ^{1}H NMR spectrum of **1a** ($CDCl_3$) (cf., δ 2.75 and 2.44 in C_6D_6). These chemical shifts indicated that the carbon atom [C(22)] bearing these protons must be attached to an sp^2-type quaternary carbon atom. Examination of Table 2 revealed that the units of **1** seriously differed from those of **2** only in the units(ii) and (ix), indicating the (C_{sp^3}) and (C_{sp^2}) atoms in the unit(ix) of **1** and **2** must be the methylene carbon [C(22)] and methyl-substituted olefinic carbon atoms [C(25)] in the unit(ii) of **1** and **2**, respectively. Thus the moiety (F) can be constructed reasonably by the units(ii), (iii), (iv), (vi), (viii), and (ix). The remaining unit is only that (v).

Careful and repeated measurement of the NOE difference spectra revealed the presence of the small NOEs between one (19β-H)[11] of two geminal protons in the unit(v) and both the protons (1β-H) (Fig. 6) and (11β-H) in the moieties (C) and (F).[12] This finding led us to presume that one of two quaternary carbon atoms of the unit(v) must be identical with the undefined one [C(10)] of the moiety (C), and another with that [C(9)] attached to the carbon bearing the relevant proton (11β-H), respectively. Thus partial structures (C) and (F), and the unit(v) must be connected straightforward as shown in Fig. 7. GEA is therefore represented favorably by formula **1**, in which the absolute configuration remains undetermined.

Structural Elucidation of GEA on the Basis of the X-Ray Diffraction Analysis. A good single crystal of GEA p-BPE (**1a**) was grown by slow evaporation from the solution in hexane and dichloromethane containing a trace amount of methanol. The crystal data follow as: $C_{41}H_{44}O_9Br_2 \cdot 1/2CH_2Cl_2$, monoclinic, space group $P2_1$, a=18.596(6), b=15.010(4), c=16.112(5) Å,

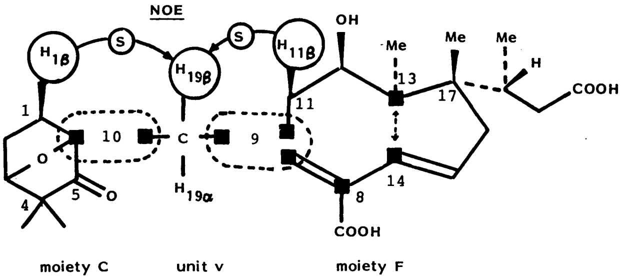


Fig. 7. A combination of the moieties (C) and (F) and the unit (v).

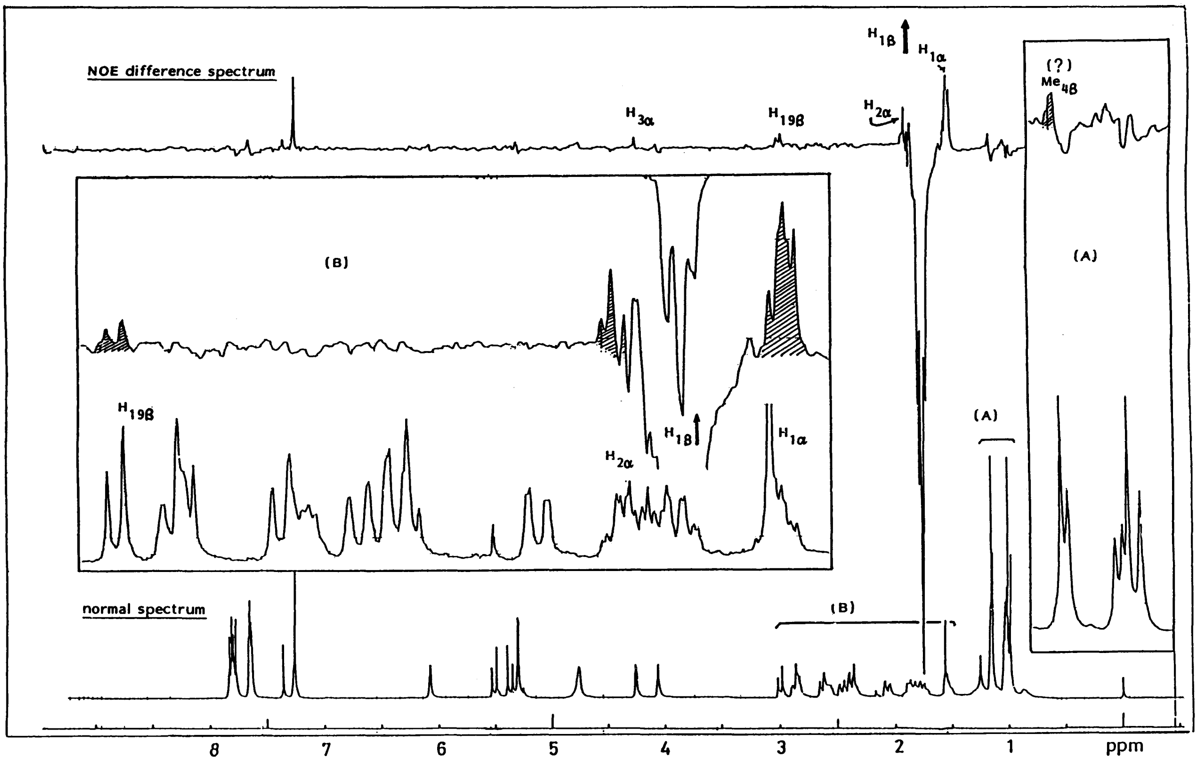
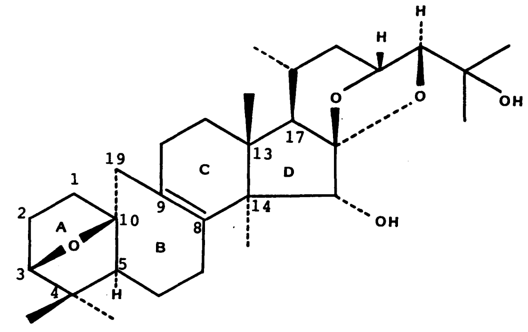


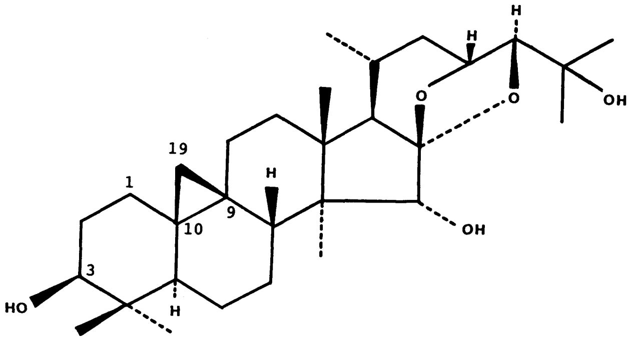
Fig. 6. The NOE difference spectrum of GEA p-BPE (**1a**) on irradiation at δ 1.76 (1β-H) (400 MHz, 0.5 mg in $CDCl_3$ and D_2O).

β=114.41(2)°, Z=4, D_c=1.432 g cm^{-3}, μ(Cu-$K\alpha$)=35.6 cm^{-1}. The intensities of 4725 independent reflections with $2\theta<120°$ were collected on an automatic, four-circle diffractometer with graphite-monochromated Cu-$K\alpha$ radiation using the ω—2θ scanning technique.[13] Since three standard reflections showed a gradual decrease in intensity with the course of data collection, the intensities were corrected for this damage to the sample as well as for the Lorenz and polarization factors. The structure was solved by the Monte Carlo direct method,[14] using the 30 strongest reflections as a starting set. An E-map based on the 58th random phase set afforded all the non-hydrogen atoms in the two ester molecules. The dichloromethane molecule was located in a difference Fourier map. After several cycles of the least-squares refinement had been carried out using the carbon atomic scattering factors for all the light atoms, the oxygen atoms were distinguished from the carbon atoms on the basis of the isotropic temperature factors and the bond distances and angles. The structure was then refined by the block-diagonal least-squares method with anisotropic thermal parameters. The absolute configuration was assigned by taking account of the anomalous dispersion of the halogen atoms for Cu-$K\alpha$ radiation. The R-factor for two enantiomeric structures, 1.106, excluded one of them at the 99.5% confidence level.[15] After the 78 hydrogen atoms had been located in a second difference Fourier map, further least-squares refinement were performed including these hydrogen atoms. The final R-value was 7.8%. These two crystallographically-independent molecules of **1a** had almost the same geometries, and one of the two is depicted in Fig. 8. The molecular structure including the absolute configuration of **1a** has thus been determined, and hence the structure of GEA is represented by formula **1**.[16]

On the basis of the assigned structure, we propose the following biogenesis for formation of GEA, shown in Fig. 9. The biogenesis, including a cycloartane, one of naturally occurring triterpenes such as acerinol[9] (**4**) and cimigenol[9] (**6**), as a



acerinol (4)



cimigenol (6)

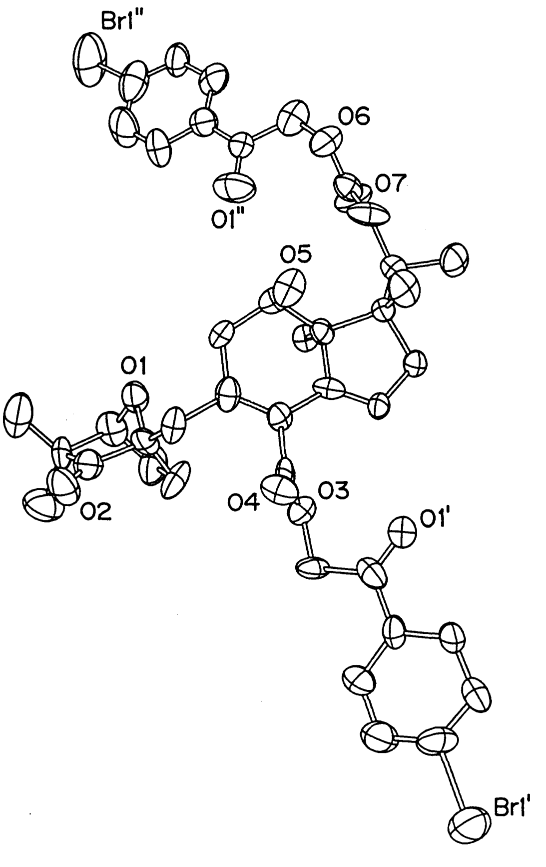


Fig. 8. The X-ray crystal structure of GEA p-BPE (**1a**).

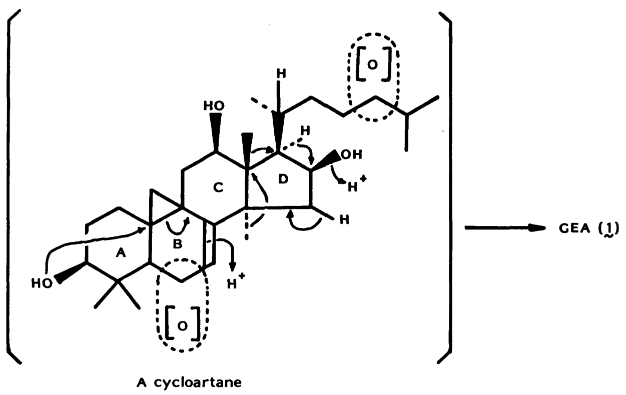


Fig. 9. A plausible biogenesis for formation of GEA (**1**).

precursor, involves (1) migration of two methyl groups in the C and D rings in a back-bone rearrangement manner, (2) cleavage of the C(9)–C(10) bond followed by formation of a 7-oxabicyclo[2.2.1]-heptane system, and (3) oxidative cleavage of the B ring and side chain with loss of one and four carbon atoms. Thus GEA (**1**) is regarded as a pentanortriterpene. These biogenetical characteristics represent the distinctive structural features of GEA. The structure with such characteristics has not been encountered previously. Only recently, isolation of a triterpene, mariesiic acid A, "having a methyl-rearranged skeleton of lanostane" from *Abies mariesii* has been reported by Hasegawa et al.[17]

Structural Elucidation of GEB. The molecular formula of GEB (**2**) was established as $C_{31}H_{42}O_9$ on the basis of the FD-MS spectrum [*m/z* 950, 952, and 954 (1:2:1)] of GEB p-BPE (**2a**) as well as the ^{1}H NMR spectra of **2a** [$CDCl_3$ (Fig. 10) and C_6D_6]. The ^{13}C NMR (Table 1) and ^{1}H NMR spectra of **2a** measured in the same manner as **1a**, indicated the presence of ten structural units(i) to (x) tabulated in Table 2. The ^{1}H NMR spectral properties, especially the difference NOEs[7,8] and coupling constants summarized in Fig. 11, of most of the protons included in these units, were essentially identical with those of the corresponding protons of **1a**. Thus structural moieties (a) including the units(i), (vii), and (x) (**2a**, IR_{max} 1750 cm^{-1}), (b) equivalent to the unit(v), (c) involving the units(iii), (iv), (vi), and (viii) [**2**, UV_{max} 252 nm (ε 9500)], and (d) containing the units(ii) and (ix), must constitute the A, B (cleaved), C and D rings, and the side chain, respectively. In view of the co-occurrence of **1** and **2** in the same plant, GEB

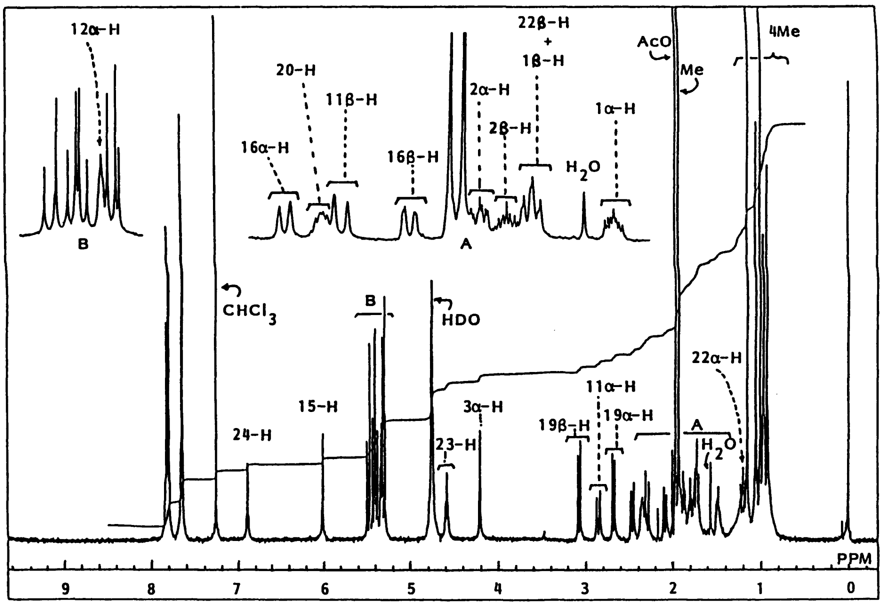


Fig. 10. The ^{1}H NMR spectrum of GEB p-BPE (**2a**) (500 MHz, 1 mg in $CDCl_3$ and D_2O).

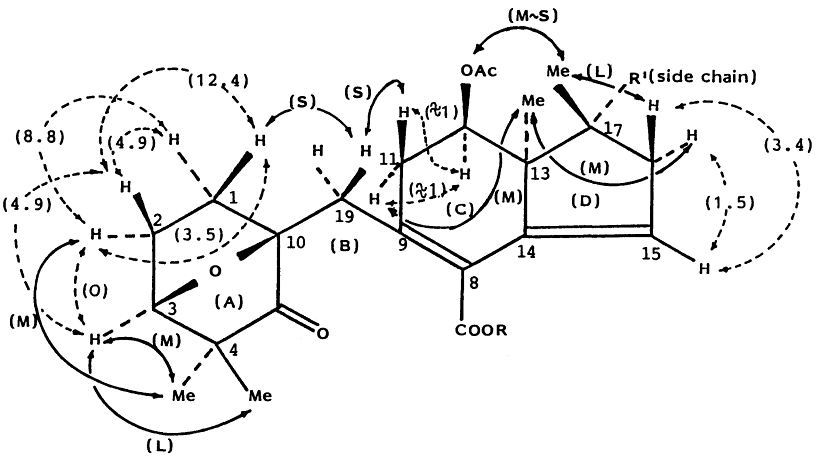


Fig. 11. The NOEs (↶↷) and coupling constants (Hz) between the protons in the A→D rings of GEB p-BPE (**2a**) ($R=CH_2COC_6H_4Br$).

must possess the same skeleton and the same absolute configuration as GEA, differing from **1** only in the substituent (OH→OAc) at C(12) and the side chain. The structure of the side chain [the units(ii) and (ix)] was elucidated on the basis of the result of conformational analysis, deduced from the difference NOEs[7,8] (Fig. 12) between the relevant protons. The result was also consistent with the following coupling constants (Hz): $J_{20\alpha,22\beta}=0$ (dihedral angle, $\theta \fallingdotseq 110^\circ$); $J_{20\alpha,22\alpha}=11.1$ ($\theta \fallingdotseq 10^\circ$); $J_{22\beta,23\alpha}=12.8$ ($\theta \fallingdotseq 160^\circ$); $J_{22\alpha,23\alpha}=2.0$ ($\theta \fallingdotseq 80^\circ$); $J_{23\alpha,24}=8.2$ ($\theta \fallingdotseq 180^\circ$); $J_{24,27}=1.2$. The conformation depicted in Fig. 12 indicates that **2a** possesses the (20*R*, 23*S*, 24*E*) configuration. GEB is therefore represented most favorably by formula **2**.

Structure Elucidation of GEC. The molecular formula of GEC (**3**) was established as $C_{29}H_{38}O_9$ on the basis of the FD-MS spectrum [*m/z* 906, 908, and 910 (1:2:1)] and the ^{1}H NMR spectra of GEC p-BPE (**3a**). The ^{13}C NMR (Table 1) and ^{1}H NMR spectra of **3a** revealed the existence of ten structural units(i) to (x) shown in Table 2. Detailed examination of the NOE difference spectra between the methyl and other protons included in these units indicated that GEC (**3**) must possess the same skeleton as GEA (**1**) with the C(16) atom substituted by an ether oxygen atom, which involves three structural moieties (a) including the units(i), (vii), and (x) (**3a**, IR_{max} 1754 cm^{-1}), (b) the unit(v), and (c) including the units(ii), (iii), (iv), (vi), and (viii) [**3**, UV_{max} 253 nm (ε 9500)], corresponding to the A, B (cleaved), and C and D rings, respectively. The (20*R*, 23*R*, 24*E*) configuration in the D ring and the side chain was deduced mainly from the difference NOEs[7,8] shown in Fig. 13 (cf., Fig. 4 in Ref. 4). The coupling and difference coupling constants (Hz) between the relevant protons were in good accord with the conformation depicted in Fig. 13: $J_{20\beta,22\beta}=6$ (dihedral angle, $\theta \fallingdotseq 35^\circ$); $J_{20\beta,22\alpha}=13$ ($\theta \fallingdotseq 155^\circ$); $J_{22\beta,22\alpha}=13$; $J_{22\beta,23\beta}=3$ ($\theta \fallingdotseq 40^\circ$); $J_{22\alpha,23\beta}=13$ ($\theta \fallingdotseq 160^\circ$); $J_{23\beta,24}=8$ ($\theta \fallingdotseq 170^\circ$); $J_{24,27}=1.2$. GEC is therefore represented best by formula **3**.

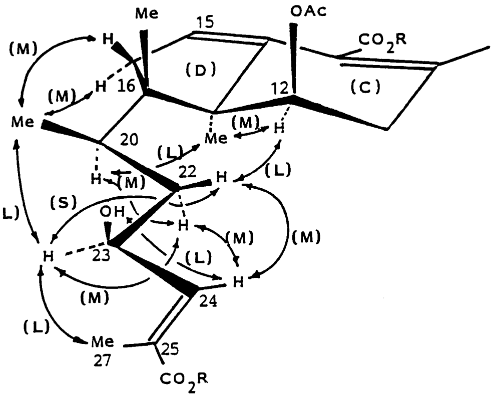


Fig. 12. The difference NOEs between the protons in the side chain of GEB p-BPE (**2a**) (R= $CH_2COC_6H_4Br$).

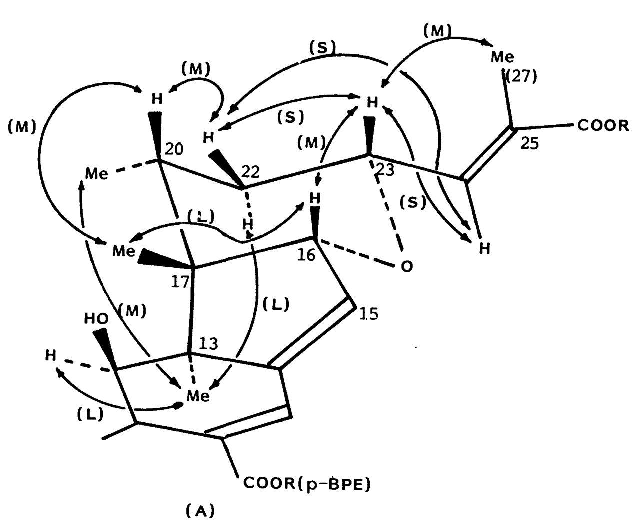


Fig. 13. The difference NOEs between the protons in the side chain of GEC p-BPE (**3a**) (R= $CH_2COC_6H_4Br$).

Experimental

The IR spectra were recorded on KBr disks with JASCO model IRA-810 and/or JEOL model JIR-100 spectrometers, and the UV spectra in methanol with a Hitachi model 124 spectrometer. The MS spectra was measured with JEOL model JMS-D300 and/or JMS-OISG-2 spectrometers. The ^{1}H NMR spectra, COSY spectra, and NOE difference spectra, and decoupling difference spectra were measured in [^{2}H]chloroform, (Merck Co., Inc. and Aldrich Chem. Co., Inc.), [$^2H_6$]benzene, and/or [$^2H_4$]methanol with JEOL model PS-100, FX-400, GX-400 and/or GX-500 spectrometers, TMS (Aldrich Chem. Co., Inc.) being used as an internal standard. The ^{13}C NMR spectra were recorded in [^{2}H]chloroform and/or [$^2H_6$]benzene with JEOL model FX-90, FX-100 and/or GX-500 spectrometers.

GEA p-BPE (1a). CD, λ_{max}^{MeOH} ($\Delta\varepsilon$) 257.5 nm (−5.6), 235.0 (−1.2), 224 (−2.0), and 198.5 (+10.6): UV (MeOH), 254.5 nm (ε 27100): IR (KBr), 1760 (sh), 1738 (s), 1704 (s), 1587 (m), 1543 (w), 1460 (w), 1422, 1377, 1231 (w), 1202, 1171, 1113, 1071 (s), 975 (s), 823 (m), and 761 (m) cm^{-1}: ^{1}H NMR (500 MHz, $CDCl_3$ and D_2O) (Fig. 1), δ=0.99 (3H, br s, 28-H=13α-CH_3), 1.02 (3H, s, 29-H=4α-CH_3), 1.04 (3H, d, J=6.5 Hz, 21-H=20α-CH_3), 1.15 (3H, br s, 18-H=17β-CH_3), 1.17 (3H, s, 30-H, 4β-CH_3), 1.54 (1H, ddd, J=12, 9, and 4 Hz, 1α-H), 1.76 (1H, ddd, J=12, 10, and 5 Hz, 1β-H), 1.84 (1H, dddd, J=12, 10, 5, and 4 Hz, 2β-H), 1.90 (1H, ddd, J=12, 9 and 5 Hz, 2α-H), 2.08 (1H, dd, J=17 and 3.5 Hz, 16β-H), 2.37 (1H, dd, J=14.5 and 11.5 Hz, 22β-H), 2.39 (1H, dd, J=18 and 1.5 Hz, 11β-H), 2.47 (1H, dd, J=17 and 1.5 Hz, 16α-H), 2.58 (1H, dq J=11.5 and 6.5 Hz, 20β-H), 2.64 (1H, br d, J=14.5 Hz, 22α-H), 2.85 (1H, d, J=14.5 Hz, 19α-H), 2.86 (1H, dd, J=18 and 3 Hz, 11α-H), 3.00 (1H, d, J=14.5 Hz, 19β-H), 4.07 (1H, dd, J=3 and 1.5 Hz, 12α-H), 4.26 (1H, d, J=5 Hz, 3α-H), and 6.08 (1H, dd, J=3.5 and 1.5 Hz, 15-H); p-BPE part, δ=5.28 and 5.33 (each 1H, ABq, J=16.5 Hz, $BrC_6H_4COCH_2$), 5.38 and 5.49 (each 1H, ABq, J=16.5 Hz, $BrC_6H_4COCH_2$), 7.65 and 7.79 (each 2H, ABq, J=8.5 Hz, $BrC_6H_4COCH_2$), 7.65 and 7.83 (each 2H, ABq, J=8.5 Hz,

$BrC_6H_4COCH_2$): ^1H NMR (500 MHz, C_6D_6) δ=0.73 (3H, s, 29-H=4α-CH_3), 1.05 (3H, br s, 28-H=13α-CH_3), 1.10 (3H, s, 30-H=4β-CH_3), 1.10 (3H, d, *J*=6.5 Hz, 21-H=20α-CH_3), 1.24 (3H, br s, 18-H=17β-CH_3), ≈1.43 and ≈1.70 (3H and 1H, each m, 1α-H to 2β-H), 1.97 (1H, dd, *J*=17 and 3.5 Hz, 16β-H), 2.34 (1H, dd, *J*=18 and 1.5 Hz, 11β-H), 2.36 (1H, dd, *J*=17 and 1.5 Hz, 16α-H), 2.44 (1H, dd, *J*=14.5 and 11.5 Hz, 22β-H), 2.75 (1H, br d, *J*=14.5, 22α-H), ≈2.75 (1H, br m, 20β-H), 2.87 (1H, dd, *J*=18 and 3 Hz, 11α-H), 3.06 (1H, d, *J*=14.5 Hz, 19α-H), 3.22 (1H, d, *J*=14.5 Hz, 19β-H), 3.78 (1H, d, *J*=5 Hz, 3α-H), 3.93 (1H, br d, *J*=7.5 Hz, 12α-H), and 6.54 (1H, br s, 15-H); p-BPE part, δ=4.72 and 4.83 (each 1H, ABq, *J*=16.5 Hz, $BrC_6H_4COCH_2$), 4.82 and 5.10 (each 1H, ABq, *J*=16.5 Hz, $BrC_6H_4COCH_2$), ≈7.06 and ≈7.19 (each 4H, m, 2×$BrC_6H_4COCH_2$): ^{13}C NMR (25 MHz, $CDCl_3$) (Table 1), p-BPE part, δ=65.58 (t), 65.93 (t), 129.0 (s), 129.1 (d), 132.0 (d), 132.7 (s), 191.0 (s), and 191.2 (s): ^{13}C NMR (25 MHz, C_6D_6) (Table 1), p-BPE part, δ=66.10 (t), 66.51 (t), 129.0 (s), 129.1 (d), 132.0 (d), 132.9 (s), 191.2 (s), and 191.2 (s): FD-MS, *m/z* 842, 840 (base), 838 (1:2:1), and 843, 841, 839 (1:2:1): CI(NH_3)-MS, *m/z* 860, 858, 856 (1:2:1), 662, 660 (1:1), 644, 642 (1:1), 624, 185, 183 (1:1), 138 (base): EI-MS, *m/z* 598, 596 (1:1) ($C_{32}H_{37}O_6Br$), 557, 555 (1:1), 511, 509 (1:1), 185, 183 (base) (1:1) (C_7H_4OBr).

GEA (1). UV (MeOH), 251.5 nm (ε 8300): IR (KBr), 3438—3380 (m), 1758 (w), 1670 (w), 1575 (vs), 1425 (s), 1411 (s), 1333 (w), 1043 (m), 1012 (m), 924 (w), 837 (w), 650 (m), and 619 (m) cm^{-1}: ^1H NMR (500 MHz, CD_3OD and D_2O), δ=1.01 (3H, d, *J*=6.7 Hz, 21-H=20α-CH_3), 1.08 and 1.18 (each br s, 28- and 18-H=13α-CH_3 and 17β-CH_3), 1.11 and 1.25 (each s, 29- and 30-H=4α-CH_3 and 4β-CH_3), ≈1.40 (4H, m, 1α-H to 2β-H), ≈2.00 (5H, m, 16β-H, 22β-H, 16α-H, 20β-H, and 22α-H), 2.48 (1H, d, *J*=14.5 Hz, 19α-H), 2.52 and 2.85 (each 1H, br d, *J*=18.9 Hz, 11β-H and 11α-H), 3.06 (1H, d, *J*=14.5 Hz, 19β-H), 4.08 (1H, br s, 12α-H), 4.39 (1H, d, *J*=4.5 Hz, 3α-H), and 5.66 (1H, br s, 15-H).

GEB p-BPE (2a). IR (KBr), 3200, 1750 (m), 1716 (s), 1579 (m), 1374, 1242 (s), 1210 (s), 1060 (m), 968 (s), 812 (m), and 747 (m) cm^{-1}: ^1H NMR (500 MHz, $CDCl_3$) (Fig. 7), δ=0.93 (3H, br s, 28-H=13α-CH_3), 0.97 (3H, d, *J*=6.7 Hz, 21-H=20α-CH_3), 1.01 (3H, s, 29-H=4α-CH_3), 1.06 (3H, br s, 18-H=17β-CH_3), 1.15 (3H, s, 30-H=4β-CH_3), 1.20 (1H, ddd, *J*=12.8, 11.1, and 2.0 Hz, 22α-H), 1.48 (1H, ddd, *J*=12.4, 8.8, and 4.9 Hz, 1α-H), 1.62 (1H, br d, *J*=3.7 Hz, D_2O-exchangeable, OH), 1.72 (2H, m, 1β-H and 22β-H), 1.80 (1H, dddd, *J*=12.4, 12.4, 4.9, and 4.9 Hz, 2β-H), 1.87 (1H, ddd, *J*=12.4, 8.8, and 3.5 Hz, 2α-H), 1.93 (3H, d, *J*=1.2 Hz, 27-H=25-CH_3), 1.97 (3H, s, $OCOCH_3$), 2.09 (1H, br dd, *J*=17.0 and 3.4 Hz, 16β-H), 2.30 (1H, br d, *J*=19.8 Hz, 11β-H), 2.35 (1H, br dq, *J*=11.1 and 6.7 Hz, 20α-H), 2.46 (1H, br d, *J*=17.0 Hz, 16α-H), 2.68 (1H, d, *J*=14.8 Hz, 19α-H), 2.86 (1H, br d, *J*=19.8 Hz, 11α-H), 3.08 (1H, d, *J*=14.8, 19β-H), 4.21 (1H, d, *J*=4.9 Hz, 3α-H), 4.60 (1H, dddd, *J*=12.8, 8.2, 3.7, and 2.0, and ddd, *J*=12.8, 8.2, and 2.0 on addition of D_2O, 23α-H), 5.33 (1H, br s, 12α-H), 6.02 (1H, br s, 15-H), 6.89 (1H, dq, *J*=8.2 and 1.2 Hz, 24-H); p-BPE part, δ=5.30 and 5.48 (each 1H, ABq, *J*=16.5 Hz, $BrC_6H_4COCH_2$), 5.40 and 5.42 (each 1H, ABq, *J*=16.5 Hz, $BrC_6H_4COCH_2$), 7.65 (4H, m, $BrC_6H_4COCH_2Br$), and 7.80 and 7.83 (each 2H, ABq, *J*=8.5 Hz, $BrC_6H_4COCH_2$): ^1H NMR (400 MHz, C_6D_6), δ=0.75 (3H, s, 29-H=3α-CH_3), 0.83 (3H, d, *J*=6.8 Hz, 21-H=20α-CH_3), 0.94 (1H, br d, *J*=4.0 Hz, D_2O-exchangeable, OH), 1.05 (3H, br s, 28-H=13α-CH_3), 1.09 (3H, s, 30-H=4β-CH_3), 1.20 (3H, br s, 18-H=17β-CH_3), 1.20 (1H, m, 22α-H), 1.45 (3H, m, 1α-H, 1β-H, and 2β-H), 1.64 (3H, s, $OCOCH_3$), 1.76 (1H, m, 2α-H), 1.90 (3H, d, *J*=1.5 Hz, 27-H=25-CH_3), 1.92 (1H, br dd, *J*=14.0 and 11.0 Hz, 22β-H), 2.06 (1H, br dd, *J*=16.6 and 3.4, 16β-H), 2.37 (1H, m, 20β-H), 2.43 (1H, br d, *J*=16.6 Hz, 16α-H), 2.48 (1H, br d, *J*=19.0 Hz, 11β-H), 2.85 (1H, d, *J*=14.6 Hz, 19α-H), 3.02 (1H, br d, *J*=19.0 Hz, 11α-H), 3.41 (1H, d, *J*=14.6 Hz, 19β-H), 3.79 (1H, br d, *J*=2.4 Hz, 3α-H), 4.27 (1H, m, 23α-H), 5.70 (1H, br s, 12α-H), 6.59 (1H, br s, 15-H); p-BPE part, δ=4.66 and 4.90 (each 1H, ABq, *J*=16.5 Hz $BrC_6H_4COCH_2$), 4.79 and 5.01 (each 1H, ABq, *J*=16.5 Hz, $BrC_6H_4COCH_2$), 7.04 and 7.15 (each 2H, ABq, *J*=8.5 Hz, $BrC_6H_4COCH_2$), 7.10 and 7.12 (each 2H, ABq, *J*=8.5 Hz, $BrC_6H_4COCH_2$): ^{13}C NMR (25 MHz, C_6D_6) (Table 1), p-BPE part, δ=66.8 (t), 127.1 (s), 129.9 (d), 132.4 (d), 134.4 (s), 135.6 (s), 191.6 (s), and 192.0 (s): FD-MS, *m/z* 954, 952 (base), 950 (1:2:1).

GEB (2). A solution (soln) of **2a** (0.3 mg) in 0.1 M[†] potassium hydroxide (KOH) in methanol (0.1 ml) was stirred at room temperature (temp) for 10 h. After being evaporated, the reaction mixture was mixed with 0.2 M hydrochloric acid (HCl) (0.2 ml) and extracted with ethyl acetate (3×0.1 ml). The ethyl acetate soln was dried and evaporated to leave an morphous residue, which was purified by preparative HPLC on a NOVA-PAK cartridge C_{18} column (8 mm×10 cm) with a UV light (254 nm) monitor, a 4:6 mixture of acetonitrile and 1% aqueous (aq) acetic acid being used as an eluent at a flow rate of 15 ml min^{-1}, giving **2** (0.17 mg), amorphous, UV (MeOH), 245 (sh, ε 8000), 252 (9500), and 260 nm (sh, 7300): IR (KBr), 3417 (m), 1740 (w), 1575 (vs), 1543 (s), 1414 (s), 1016 (w), 651 (m), and 620 (w) cm^{-1}: ^1H NMR (500 MHz, CD_3OD) δ=0.96 (3H, d, *J*=6.4 Hz, 21-H=20α-CH_3), 0.99 and 1.15 (each 3H, s, 29-H and 30-H=4α-CH_3 and 4β-CH_3), 1.01 and 1.11 (each 3H, br s, 28-H and 18-H=13α-CH_3 and 17β-CH_3), 1.25 (1H, m, 22α-H), 1.37 (1H, m, 1α-H), 1.78 (2H, m, 1β-H and 22β-H), 1.84 (3H, br s, 27-H=25-CH_3), 1.86 (1H, m, 2β-H), 1.88 (3H, s, $OCOCH_3$), 1.93 (1H, ddd, *J*=9, 9, and 3 Hz, 2β-H), 1.95 (1H, dd, *J*=18 and 4 Hz, 16β-H), 2.03 (1H, br d, *J*=19 Hz, 11β-H), 2.32 (1H, d, *J*=15 Hz, 19α-H), 2.33 (1H, m, 20β-H), 2.42 (1H, d, *J*=18 Hz, 16α-H), 2.84 (1H, br d, *J*=19 Hz, 11α-H), 3.03 (1H, d, *J*=15 Hz, 19β-H), 4.03 (1H, br s, 12α-H), 4.24 (1H, d, *J*=5 Hz, 3α-H), 4.47 (1H, ddd, *J*=11, 9, and 2 Hz, 23α-H), 5.58 (1H, br s, 15-H), and 6.43 (1H, m, 24-H).

Deacetylglycinoeclepin B (2b). A soln of **2** (0.1 mg) in 5% sodium methoxide in methanol (0.1 ml) was stirred at 70 °C for 10 h. The mixture was evaporated, acidified with 0.2 M HCl (0.2 ml), and extracted with ethyl acetate (3×0.1 ml). The ethyl acetate soln was dried and evaporated to leave an oily residue, which was purified by preparative HPLC on a NOVA-PAK cartridge C_{18} column (8 mm×10 cm), a 4:6 mixture of acetonitrile and 1% aq acetic acid being used as an eluent at a flow rate of 1.5 ml min^{-1}, to give **2b** (0.1 mg), amorphous. UV (MeOH), 242 (sh, ε 9500), 250 (9900), and 260 nm (sh, 6900): IR (KBr), 3438—3380 (m), 1751 (w), 1576 (vs), 1545 (s), 1414 (vs), 1016 (w), 652 (m), and 621 (w) cm^{-1}: ^1H NMR (500 MHz, CD_3OD), δ=0.92 (3H, d, *J*=6.8 Hz, 21-H=20α-CH_3), 0.95 and 1.11 (each 3H, s, 29-H and 30-H=4α-

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[†] 1 M=1 mol dm^{-3}.

CH$_3$ and 4β-CH$_3$), 0.96 and 1.06 (each 3H, br s, 28-H and 18-H=13α-CH$_3$ and 17β-CH$_3$), 1.80 (3H, br s, 27-H=25-CH$_3$), 1.98 (1H, br d, *J*=18 Hz, 11β-H), 2.27 (1H, d, *J*=14 Hz, 19α-H), 2.30 (1H, m, 20β-H), 3.38 (1H, br d, *J*=16 Hz, 16α-H), 2.80 (1H, br d, *J*=18 Hz, 11α-H), 2.99 (1H, d, *J*=14 Hz, 19β-H), 3.99 (1H, m, 12α-H), 4.20 (1H, d, *J*=5 Hz, 3α-H), 4.43 (1H, m, 23α-H), and 5.54 (1H, br s, 15-H).

GEC p-BPE (3a). ^{1}H NMR (500 MHz, CDCl$_3$ and D$_2$O), δ=1.01 (3H, s, 29-H=4α-CH$_3$), 1.16 (3H, s, 30-H=4β-CH$_3$), 1.25 (3H, br s, 18-H=17β-CH$_3$), 1.29 (3H, d, *J*=7 Hz, 21-H=20α-CH$_3$), 1.37 (3H, br s, 18-H=13α-CH$_3$), 1.48 (1H, ddd, *J*=13, 6, and 3 Hz, 22β-H), 1.54 (1H, m, 1α-H), 1.75 (1H, ddd, *J*=12, 12, and 3.5 Hz, 1β-H), 1.77 (1H, ddd, *J*=13, 13, and 13 Hz, 22α-H), 1.82 (1H, dddd, *J*=12, 12, 5, and 5 Hz, 2β-H), 1.90 (1H, ddd, *J*=12, 9, and 4 Hz, 2α-H), 1.94 (3H, d, *J*=1.5 Hz, 27-H=25-CH$_3$), 2.03 (1H, m, 20β-H), 2.40 (1H, dd, *J*=19 and 1.5 Hz, 11β-H), 2.89 (1H, d, *J*=14.5 Hz, 19α-H), 2.94 (1H, br d, *J*=19 Hz, 11α-H), 3.08 (1H, d, *J*=14.5 Hz, 19β-H), 4.00 (1H, d, *J*=3.5 Hz, 16β-H), 4.08 (1H, br s, 12α-H), 4.25 (1H, d, *J*=5 Hz, 3α-H), 4.88 (1H, ddd, *J*=13, 8, and 3 Hz, 23β-H), 6.22 (1H, d, *J*=3.5 Hz, 15-H), and 6.88 (1H, dq, *J*=8 and 1.5 Hz, 24-H); p-BPE part, δ=5.28 and 5.35 (each 1H, ABq, *J*=16.5 Hz, BrC$_6$H$_4$COC*H*$_2$), 5.36 and 5.51 (each 1H, ABq, *J*=16.5 Hz, BrC$_6$H$_4$COC*H*$_2$), 7.63 and 7.65 (each 2H, ABq, *J*=8.5 Hz, BrC$_6$*H*$_4$COCH$_2$), 7.78 and 7.81 (each 2H, ABq, *J*=8.5 Hz, BrC$_6$*H*$_4$COCH$_2$): ^{1}H NMR (500 MHz, C$_6$D$_6$), δ=0.85 and 1.48 (each 3H, s, 29-H and 30-H=4α-CH$_3$ and 4β-CH$_3$), 1.19 (3H, d, *J*=7 Hz, 21-H=20α-CH$_3$), 1.21 and 1.44 (each 3H, br s, 28-H and 18-H=13α-CH$_3$ and 17β-CH$_3$), 1.25 (1H, ddd, *J*=13, 6, and 3 Hz, 22β-H), 1.39 (1H, d, *J*=7.5 Hz, OH), ≈1.48 (3H, m, 1α-H, 1β-H, and 2β-H), 1.70 (1H, m, 22α-H), 1.81 (1H, m, 20β-H), 1.85 (1H, m, 2α-H), 2.08 (3H, d, *J*=1.5 Hz, 27-H=25-CH$_3$), 2.42 (1H, br d, *J*=19 Hz, 11β-H), 3.06 (1H, br d, *J*=19 Hz, 11α-H), 3.18 (1H, d, *J*=14.5 Hz, 19α-H), 3.44 (1H, d, *J*=14.5 Hz, 19β-H), 3.90 (1H, d, *J*=4 Hz, 3α-H), 4.05 (1H, br d, *J*=7.5 Hz, 12α-H), 4.09 (1H, d, *J*=3 Hz, 16β-H), 4.24 (1H, ddd, *J*=13, 8, and 3 Hz, 23β-H), 6.90 (1H, d, *J*=3 Hz, 15-H), and 7.42 (1H, dq, *J*=8 and 1.5 Hz, 24-H); p-BPE part, δ=4.89 and 5.17 (each 1H, ABq, *J*=16.5 Hz, BrC$_6$H$_4$COC*H*$_2$), 4.90 and 4.98 (each 1H, ABq, *J*=16.5 Hz, BrC$_6$H$_4$COC*H*$_2$), 7.15 and 7.16 (each 2H, ABq, *J*=8.5 Hz, BrC$_6$*H*$_4$COCH$_2$), 7.26 and 7.28 (each 2H, ABq, *J*=8.5 Hz, BrC$_6$*H*$_4$COCH$_2$): ^{13}C NMR (125 MHz, C$_6$D$_6$), (Table 1), p-BPE part, δ=66.1 (t), 66.2 (t), 129.36 (d), 129.44 (d), 132.0 (d), 133.2 (s), 133.4 (s), 190.77 (s), 190.83 (s): FD-MS, *m/z* 910, 908 (base), 906 (1:2:1).

GEC (3). Compound **3a** (0.5 mg) was hydrolyzed with 0.1 M KOH in methanol at room temp for 10 h. The reaction mixture was worked up as usual to yield an amorphous residue, which was purified by preparative HPLC as described above to give **3** (0.26 mg), amorphous. UV (MeOH), 253 nm (ε 9500): IR (KBr), 3375 (m), 1761 (m), 1572 (vs), 1414 (vs), 1055 (m), 1018 (m), and 654 (w) cm^{-1}: ^{1}H NMR (400 MHz, CD$_3$OD), δ=0.99 and 1.15 (each 3H, s, 29-H and 30-H=4α-CH$_3$ and 4β-CH$_3$), 1.23 and 1.29 (each 3H, br s, 28-H and 18-H=13α-CH$_3$ and 17β-CH$_3$), 1.28 (3H, d, *J*=6.5 Hz, 21-H=20α-CH$_3$), 1.89 (3H, br s, 27-H=25-CH$_3$), 2.01 (1H, m, 20β-H), 2.06 (1H, br d, *J*=18.5 Hz, 11β-H), 2.33 (1H, br d, *J*=14 Hz, 19α-H), 2.88 (1H, br d, *J*=18.5 Hz, 11α-H), 3.05 (1H, br d, *J*=14 Hz, 19β-H), 3.90 (1H, br s, 16β-H), 3.93 (1H, br s, 12α-H), 4.24 (2H, m, 3α-H and 23β-H), 5.75 (1H, br s, 15-H), and 6.37 (1H, m, 24-H).

1-Bromomethyl-3,3-dimethyl-7-oxabicyclo[2.2.1]heptan-2-one (5). (Scheme 1). a) A soln of α-chloroacrylonitrile (18.5 g, 0.22 mol), 2,4-dimethyl-1,3-pentadiene (10.5 g, 0.11 mol), and 2,6-di-*t*-butyl-4-methylphenol (BHT, 500 mg) in benzene (100 ml) was stirred under reflux for 7 h. The reaction mixture was evaporated and then distilled under reduced pressure to give 1-chloro-2,2,4-trimethyl-3-cyclohexene-1-carbonitrile (**5a**) (12.1 g), bp 108—113 °C (12 Torr) (1 Torr=133.322 Pa). The undistilled residue was submitted to chromatography over silica gel with hexane–benzene (10:1) to afford an additional amount (4.0 g) of **5a**. The total yield of **5a** amounted to 80%. **5a**: ^{1}H NMR (100 MHz, CCl$_4$), δ=1.17, 1.30, and 1.69 (each 3H, s), and 5.11 (1H, br s): MS, *m/z* 185 and 183 (1:3, M$^+$), 132 and 96 (base).

b) To a soln of copper(II) sulfate (68.0 g as 5H$_2$O, 0.272 mol) in water (250 ml) was added zinc powder at 0 °C. The mixture was stirred at the temp for 10 min and filtered to give zinc-copper couples (Zn–Cu), which were washed with water and ethanol. To a soln of **5a** (25.0 g, 0.136 mol) in 80% aq ethanol (750 ml) were added the couples. The mixture was stirred under reflux for 15 h, when precipitates (ppts) appeared, and were then collected by filtration and washed with methanol and ethyl acetate repeatedly. All the organic solns were combined, evaporated, diluted with saturated brine, and extracted with ethyl acetate. The ethyl acetate soln was washed with water and brine, dried and evaporated to leave an oily residue, which was distilled under reduced pressure to afford 2,2,4-trimethyl-3-cyclohexene-1-carbonitrile (**5b**) (19.6 g, 97%), bp 122—123 °C (15 Torr): IR (neat), 2220 and 1460 cm^{-1}: ^{1}H NMR (100 MHz, CCl$_4$), δ=1.11 (6H, s), 1.63 (3H, s), and 5.08 (1H, br s): MS, *m/z* 149 (M$^+$), 134, 107, and 96 (base).

c) To a soln of diisopropylamine (12.9 ml, 92 mmol) in tetrahydrofuran (THF, 100 ml) was added butyllithium [BuLi, 1.6 M hexane soln (57.5 ml, 92 mmol)] at 0 °C, and the mixture was stirred for 30 min at 0 °C. To the mixture cooled at −78 °C was added **5b** (12.4 g, 83 mmol) in THF (60 ml) during a period of 1 h, and the mixture was then stirred at the temp for 1 h. Into the mixture was bubbled oxygen gas at −78 °C for 1 h, and was then added triethyl phosphite (15 ml, 87 mmol). The whole mixture was stirred at −78 °C for 15 min and then at 0 °C for 1.5 h. The reaction mixture was diluted with water and extracted with diethyl ether. The ether soln was washed with water and brine, dried, and evaporated to leave an oily residue, which was distilled in vacuo to give 2,2,4-trimethyl-3-cyclohexen-1-one (4.1 g). The undistilled residue was separated by chromatography over silica gel (130 g) with hexane–benzene (1:1) to yield an additional amount of the ketone (3.4 g): ^{1}H NMR (100 MHz, CCl$_4$), δ=1.07 (6H, s), 1.72 (3H, s), and 5.20 (1H, br s). The combined ketone was reduced without further purification.

A soln of the ketone (7.5 g, 54 mmol) in methanol (100 ml) was stirred with sodium borohydride (2.0 g, 54 mmol) at 0 °C for 1 h. After being quenched with acetic acid and neutralized with saturated aq sodium hydrogencarbonate (NaHCO$_3$), the reaction mixture was concentrated, diluted with water, and extracted with ether and then with ethyl acetate. The combined extracts were washed with saturated brine, dried, and evaporated to leave an oily residue, which was purified by distillation to give 2,2,4-trimethyl-3-cyclohexen-1-ol (**5c**) (7.17 g, 95%), bp 48—49 °C (0.35 Torr):

^{1}H NMR (100 MHz, CDCl$_3$), δ=0.95, 1.00, and 1.62 (each 3H, s), 3.53 (1H, dd, J=7 and 4 Hz), and 5.06 (1H, br s).

d) To a soln of **5c** (4.7 g, 33.6 mmol) and bis(acetylacetonato)oxovanadium(IV) [VO(acac)$_2$, 10 mg, 0.035 mmol] in benzene (100 ml) was added dropwise *t*-butyl hydroperoxide (a 70% soln in water, 8.6 g, 67 mmol), which had been dried azeotropically with benzene, in benzene under stirring. The mixture was stirred at room temp for 10 h, and quenched by addition of aq sodium thiosulfate (Na$_2$S$_2$O$_3$). The aq soln was separated and extracted with ether. The benzene and ether solns were combined, washed with aq NaHCO$_3$ and brine, dried, and evaporated to leave an oily residue which was purified by chromatography over silica gel (100 g) with benzene to give 2,2,4-trimethyl-*c*-3,*c*-4-epoxy-*r*-1-cyclohexanol (**5d**) (4.85 g, 93%): ^{1}H NMR (100 MHz, CDCl$_3$), δ=1.04, 1.17, and 1.32 (each 3H, s), 2.73 (1 h, br s), and 3.30 (1H, t, J=4 Hz): MS, m/z 156 (M$^+$), 138, 123, 100, and 44 (base).

e) A soln of **5d** (400 mg, 2.56 mmol) and ethyl vinyl ether (1 ml, 10.5 mmol) in dichloromethane (20 ml) was stirred with pyridinium *p*-toluenesulfonate (PPTS, 64 mg, 0.26 mmol) at room temp for 15 h. The soln was diluted with ether, washed with aq NaHCO$_3$ and brine, dried, and evaported to leave an oily residue, which was purified by chromatography over silica gel (20 g) with benzene–ethyl acetate (10:1) to give 2-ethoxyethyl ether (**5e**) of **5d** (0.55 g, 94%): IR (neat), 1380, 1135, 1100, 1085, 1060, and 1050 cm^{-1}: ^{1}H NMR (100 MHz CCl$_4$), δ=0.93—1.24 (15H, m), 2.35 (1H, br s), 3.40(3H, m), and 4.55 (1H, m). Compound **5e** was a diastereoisomeric mixture and showed complex signals in the NMR spectrum.

f) To a soln of diethylamine (0.1 ml, 1.0 mmol) in ether (2 ml) was added BuLi [1.6 M hexane soln (0.65 ml, 1.0 mmol)] at 0 °C, and the mixture was stirred at the temp for 30 min. To this soln was added hexamethylphosphoric triamide (HMPA, 0.17 ml, 1.0 mmol) and then **5e** (114 mg, 0.5 mmol) in ether. The whole mixture was stirred under reflux for 20 h, cooled, poured into aq ammonium chloride (NH$_4$Cl), and extracted with ether repeatedly. The ether soln was washed with aq NaHCO$_3$ and brine, dried, and evaporated to leave an oily residue, which was separated by chromatography over silica gel (8 g) with benzene–ethyl acetate (10:1) to give a diastereoisomeric mixture (51 mg, 45%) of 2-ethoxyethyl ethers of 2,2-dimethyl-4-methylene-*c*-3,*r*-1-cyclohexanediol and of 2,2,4-trimethyl-4-cyclohexene-*c*-3,*r*-1-diol: IR (neat), 3490, 3040, 1660, 1450, 1380, 1130, 1095, 1025, 990, and 930 cm^{-1}: ^{1}H NMR (100 MHz, CDCl$_3$), δ=1.82 (3/2H, br s), 4.68 (1H, m), 4.82 (2/2H, br s), and 5.24 (1/2H, m). The mixture was used for the next reaction without further separation.

A soln of the mixture of the olefinic alcohols (45 mg, 0.20 mmol) in pyridine (2 ml) was treated with acetic anhydride (0.2 ml, 2.0 mmol) under stirring at room temp for 15 h. The reaction mixture was diluted with ether, washed with dil HCl, aq NaHCO$_3$ and brine, dried, and evaporated to leave an oily mixture of the acetates. This acetate mixture in THF (2 ml) was treated with 2 M HCl (0.3 ml) under stirring at room temp for 1 h. The mixture was worked up as usual to give an oily residue, which was separated by chromatography over silica gel (3 g) with benzene–ethyl acetate (5:1) to afford a mixture (30 mg, 77%) of 2,2-dimethyl-6-methylene-*c*-3-hydroxy-*r*-1-cyclohexyl acetate (**5f**) and 2,2,6-trimethyl-*c*-3-hydroxy-5-cyclohexane-*r*-1-yl acetate (**5f′**): IR (neat), 3490, 3090, 1740, 1660, 1445, 1370, 1260, 1110, 990, and 920 cm^{-1}: ^{1}H NMR (100 MHz, CDCl$_3$), δ=2.09 and 2.12 (total 3H, each s), and 3.50 (1H, m). The mixture was used for the next reaction without further separation.

g) A soln (30 mg, 0.15 mmol) of **5f** and **5f′** in acetonitrile (5 ml) was treated with N-bromosuccinimide (35 mg, 0.20 mmol) under stirring at room temp for 10 h. The mixture was diluted with ether, washed with aq NaHCO$_3$ and brine, dried, and evaporated to leave a residue, which was submitted to chromatography over silica gel (3 g) with benzene–ethyl acetate (5:1) to give 6-bromomethyl-2,2-dimethyl-*c*-3,*c*-6-epoxy-*r*-1-cyclohexyl acetate (**5g**) (14 mg, 33%) and the unreacted olefin (**5f′**) (10 mg, 33%). **5g**, mp 70—71.5 °C: IR (CHCl$_3$), 1740, 1385, 1260, 1060, and 1020 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$), δ=0.95 and 1.15 (each 3H, s), 2.02 (3H, s), 3.55 and 3.69 (each 1H, ABq, J=10.1 Hz), 4.01 (1H, d, J=4.6 Hz), and 4.61 (1H, s): MS, m/z 278, 276 (M$^+$), 235, 233, 183, and 154. Found: m/z 278.0262 and 276.0398. Calcd for C$_{11}$H$_{17}$O$_3$Br: M, 278.0340 and 276.0362. **5f′**, oil: ^{1}H NMR (100 MHz, CDCl$_3$), δ=0.96 and 0.99 (each 3H, s), 1.63 (3H, d, J=2 Hz), 2.12 (3H, s), 3.54 (1H, t, J=5 Hz), 5.48 (1H, br s), and 5.53 (1H, m).

h) A soln of **5g** (11 mg, 0.04 mmol) in an 8:1 mixture of methanol and water was treated with potassium carbonate (10 mg, 0.06 mmol) at 0 to 15 °C for 12 h. The soln was evaporated, mixed with water, and extracted with ether. The ether soln was worked up as usual to give an oily, crude alcohol, which was dissolved in acetone (4 ml). The acetone soln was treated with Jones' reagent (5 drops) at 0 °C under stirring for 30 min. The reaction mixture was quenched with isopropyl alcohol, diluted with water, and extracted with ether. The ether soln was worked up as usual to leave an oily residue, which was purified by chromatography over silica gel (3 g) with hexane–ethyl acetate (9:1) to give **5** (9.2 mg, 99%), oil: IR (neat), 1760, 1470, 1390, 1245, and 1020 cm^{-1}: ^{1}H NMR (400 MHz, CDCl$_3$), δ=1.06 and 1.23 (each 3H, s), 3.66 and 3.78 (each 1H, ABq, J=11.7 Hz), and 4.34 (1H, d, J=3.9 Hz): ^{13}C NMR (25 MHz, CDCl$_3$), δ=19.73 and 22.43 (each q), 25.83, 28.42, and 28.77 (each t), 49.61 (s), 84.01 (d), 87.30 (s), and 214.65 (s): MS, m/z 234, 232 (M$^+$), 206, 204, 125 (base), and 107. Found: m/z 234.0060 and 232.0067. Calcd for C$_9$H$_{13}$O$_2$Br: M, 234.0077 and 232.0097.

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11) The methyl groups and hydrogen atoms included in the B ring and side chains of **1a**, **2a**, and **3a** were tentatively assigned α or β on the basis of Figs. 5, 7, 12, and 13.

12) The small NOEs of the proton (19β-H) were observed clearly on irradiation of those (1β-H) and (11β-H), but any NOE of the latter protons (1β-H and 11β-H) was not detected on irradiation of the former (19β-H).

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