Conformational Analysis of Halochamigrene Derivatives by ^{1}H Dynamic Nuclear Magnetic Resonance Experiments and Molecular-mechanics Calculations. An Unusually High Barrier for the 6-Methylenecyclohex-2-en-1-one Ring Inversion

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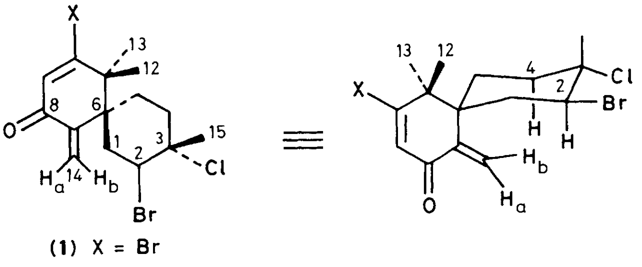
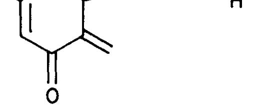
2,10-Dibromo-3-chlorochamigra-7(14),9-dien-8-one (**1**), isolated from the red alga *Laurencia nipponica*, is found as an equilibrium mixture of two stable conformational isomers the structure of which has been elucidated by ^{1}H n.m.r. experiments. The ratio of the two stable conformers is 70:30 and from this, the Gibbs free energy (ΔG°) has been determined as 2.11 kJ mol^{-1}. A conformational equilibrium of 2-bromo-3-chlorochamigra-7(14),9-dien-8-one (**2**) has also been observed. The strain-energy difference (ΔH°), calculated using molecular-mechanics calculations, between the two stable conformers of (**1**) and between those of (**2**) are 2.35 and 2.81 kJ mol^{-1}, respectively, and the variation in stability of each of the two conformers of (**1**) and (**2**) is attributed mainly to the dipole–dipole interaction energy. Using ^{1}H dynamic n.m.r. spectroscopy, the thermodynamical parameters of conformational exchange of (**1**) have been determined as $\Delta G^{\ddagger}_{313}$ = 76 kJ mol^{-1}, $\Delta H^{\ddagger}$ = 50 kJ mol^{-1}, and $\Delta S^{\ddagger}$ = −84 J mol^{-1} K^{-1}. The unusually high barrier is caused by the hindered rotation of the C(6)–C(11) axis, and to a large extent, by the non-bonded interaction between the two axial protons, 2-H and 4-H_{ax}, and an exomethylene proton, 14-H_b.

In our previous papers,[1,2] we suggested that 2,10-dibromo-3-chlorochamigra-7(14),9-dien-8-one (**1**),† isolated from the red alga *Laurencia nipponica*, exists as an equilibrium mixture of two stable conformational isomers, and its 10-debromo derivative (**2**)† exhibited the same phenomenon. The cyclohexadienone moiety must undergo rapid inversion as it was observed as a single species by n.m.r. spectroscopy at room temperature. However, the cyclohexadienone derivatives (**1**) and (**2**) were unexpectedly found as a conformational mixture. This observation suggested that an unusually high barrier was present between the two stable conformers. This novel phenomenon prompted us to undertake a conformational analysis of those sesquiterpenes using ^{1}H n.m.r. experiments, computer-aided line-shape simulation methods, and molecular-mechanics calculations.



(**1**) X = Br
(**2**) X = H



(**3**)

The ^{1}H n.m.r. spectra of (**1**) measured at 293 K in [$^{2}H_8$]toluene showed several pairs of signals, and when measured at 373 K, each pair of signals coalesced to a single one (Figure 1). The spectral change with varying temperature was reproducible. The intensity ratio of a pair of signals was observed as 70:30 from which the experimental Gibbs free energy (ΔG°) was determined as 2.11 kJ mol^{-1}. The coupling constants, $J_{1ax,2}$ 13 Hz, $J_{1eq,2}$ 5 Hz, $J_{4ax,5ax}$ 13 Hz, $J_{4ax,5eq}$ 4 Hz, $J_{4eq,5ax}$ 3.4 Hz, and $J_{4eq,5eq}$ 4 Hz for the major species and 13, 4, 14, 4, 5, and 4 Hz respectively for the minor one, revealed that the B rings of both conformations assumed the same typical chair form such that the two halogen atoms were both equatorially disposed. These B-ring conformations were supported by long-range W-couplings observed between 1-H_{eq} and 5-H_{eq}, 2-H and 15-H_3, and 4-H_{ax} and 15-H_3. Two plausible stable conformations of the dienone (**1**) involve the B ring in the typical chair form combined with the A ring which is a potentially flexible cyclohexadienone able to adopt two conformations. In the case of the major isomer, n.O.e.s were observed between 12-H_3 and 1-H_{eq}, 12-H_3 and 14-H_a, 12-H_3 and 14-H_b, 13-H_3 and 1-H_{ax}, 13-H_3 and 5-H_{ax}, and 2-H and 14-H_b. In the case of the minor conformer, n.O.e.s were observed between 12-H_3 and 1-H_{ax} and 12-H_3 and 5-H_{ax}. The above n.O.e.s strongly indicate that a rotation about the single bond, C(6)–C(11), separated two stable conformers which were the *gauche* forms about the *gem*-dimethyl groups and two methylene groups of C-(1) and C-(5). Hence the more stable conformer was represented as (**1a**), and the other as (**1b**). Molecular-mechanics calculations using Allinger's force field

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† Compounds (**1**) and (**2**) exhibited cytotoxic activity at almost identical concentration. The cytotoxicity values (IC_{50}) were *ca.* 5 μg ml^{-1} against MCF-7 human mammary carcinoma cells and 10–20 μg ml^{-1} against L1210 and P388 mouse leukaemia cells. The enantiomer of (**2**), isolated from the Okinawan sea hare, *Aplysia dactylomela*,[3] also showed cytotoxic activity at almost the same concentration as (**1**) and (**2**) (Prof. T. Higa, personal communication).

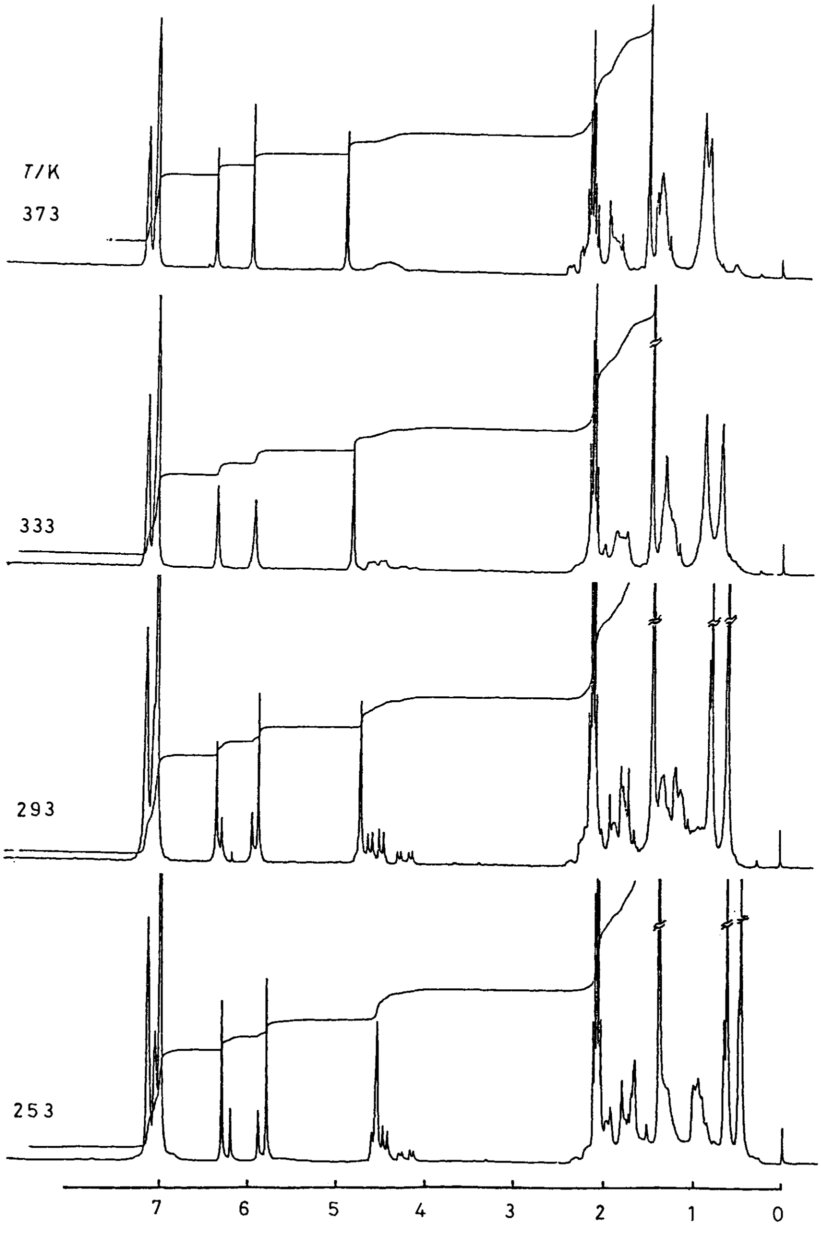


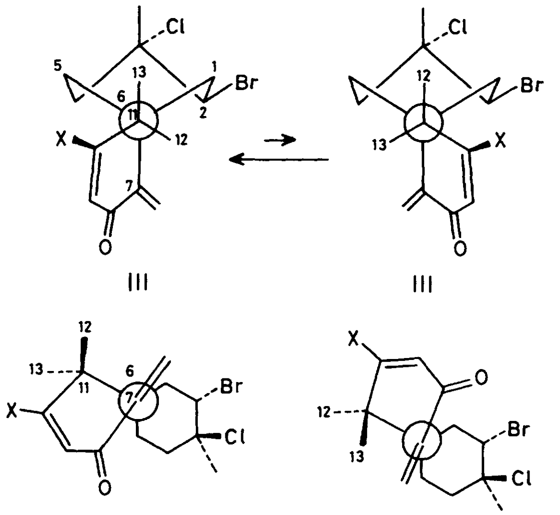
Figure 1. Temperature dependence of the ^1H n.m.r. spectra of (**1**) in [$^2H_8$]toluene

MMI/MMPI[4] were performed on the two stable conformations of (**1**). The difference in strain energy (ΔH°) between the major and minor conformers was calculated to be 2.35 kJ mol^{-1}. On the assumption that the entropy difference (ΔS°) is negligible, the Gibbs free energy is the same as the enthalpy, namely 2.35 kJ mol^{-1}. Therefore the calculated conformer ratio is 72:28 which is consistent with the observed ratio. For the comparatively more stable conformer, the dihedral angles at C(1)–C(6)–C(11)–C(12) and at C(1)–C(6)–C(7)–C(14) were estimated to be −57.87 and 17.13°, respectively, those of the other conformer were 55.05 and −132.55° (Table). The calculated conformation for the major species was consistent with (**1a**), and that for the minor conformer was consistent with (**1b**). The 10-debromo derivative (**2**) exhibited a distinct temperature dependence and was observed as two stable conformers which equilibrated slowly at room temperature as with (**1**). The difference in strain energy for these two conformers was determined, by molecular-mechanics calculations, to be 2.81 kJ mol^{-1}. The ratio of conformers was also calculated to be 74:26, coincidental with that observed in the ^1H n.m.r. spectra. The two stable conformations of (**2**) were closely similar to those of (**1**). The more stable isomer was shown to be (**2a**), and the other to be (**2b**). In addition, the conformation that exists in the crystalline state of an enantiomer of (**2**)[3] was very similar to the comparatively stable conformations of both (**1**) and (**2**). The ^1H n.m.r. spectra of (**1**) were simulated using a computer-aided line-shape equation for a two-state model.[5] The exchange rate constants were determined by the comparison between the experimental and simulated spectra (Figure 2). The plots of ln kT^{-1} against T^{-1} (k = rate constant, T = absolute temperature) of (**1**) was linear in the range 313–333 K and the activation parameters were determined using the Eyring equation; $\Delta G^{\ddagger}_{313}$ = 76 kJ mol^{-1}, $\Delta H^{\ddagger}$ = 50 kJ mol^{-1}, and $\Delta S^{\ddagger}$ = −84 J mol^{-1} K^{-1}. In order to estimate those activition parameters, ^1H

Table. Selected dihedral angles of (**1**) and (**2**) obtained by molecular mechanics calculations

Dihedral angle	Species (**1a**)	(**1b**)	(**2a**)	(**2b**)
1–2–3–4	−50.28	−52.37	−50.45	−53.22
2–3–4–5	54.26	51.75	54.20	51.92
3–4–5–6	−61.61	−56.51	−61.61	−55.80
4–5–6–1	54.89	52.40	55.38	51.69
5–6–1–2	−51.73	−54.40	−51.99	−53.99
6–1–2–3	53.95	59.06	54.02	59.41
6–7–8–9	−41.54	41.71	−38.28	37.27
7–8–9–10	4.42	−4.65	−0.93	1.49
8–9–10–11	3.69	−3.65	8.24	−8.03
9–10–11–6	22.42	−22.14	21.21	−21.76
10–11–6–7	−53.00	52.66	−53.85	53.99
11–6–7–8	66.70	−66.63	67.00	−66.25
7–6–11–12	64.85	173.10	63.82	172.83
7–6–11–13	−173.16	−65.01	−172.69	−63.62
9–10–11–12	−98.52	−145.14	−100.21	−146.06
9–10–11–13	145.19	98.56	145.34	99.60
O[a]–8–9–10	−176.50	176.16	−179.86	−179.23
O[a]–8–7–6	139.38	−139.09	140.64	−142.00
O[a]–8–7–14	−45.22	46.08	−44.18	42.99
9–8–7–14	133.86	−133.12	136.90	−137.73
11–6–7–14	−108.00	107.42	−107.49	108.04
1–6–11–12	−57.87	55.05	−59.47	54.93
1–6–11–13	64.12	176.94	64.02	178.48
5–6–11–12	−177.36	−64.18	−178.66	−64.09
5–6–11–13	−55.37	57.71	−55.17	59.46
1–6–7–14	17.13	−132.55	17.10	−131.57
5–6–7–14	131.90	−17.90	132.41	−16.05

[a] Carbonyl oxygen.



(**1a**) X = Br (**1b**)
(**2a**) X = H (**2b**)

dynamic n.m.r. spectra of (**3**), the 2-debromo-3-dechloro 2-ene derivative of (**1**), were analysed in the same way as (**1**), and the thermodynamical parameters of (**3**) were determined to be $\Delta G^{\ddagger}_{183} \leqslant 42$ kJ mol^{-1} and $\Delta H^{\ddagger} \leqslant 12$ kJ mol^{-1}. An approximate activation enthalpy for inversion of ring A of (**1**) was arrived at from three factors: (*a*) the hindered rotation about the single bond C(6)–C(11); (*b*) the non-bonded interaction between two axial protons, 2-H and 4-H$_{ax}$, and one of the exomethylene protons, 14-H$_b$; (*c*) the resonance effects on two conjugated enone systems, C(14)=C(7)–C(8)=O and C(10)=C(9)–C(8)=O. The activation enthalpy of (**3**) was due to two factors, (*a*) and (*c*), therefore the difference in activation enthalpy between (**1**) and (**3**) was derived from factor (*b*), and was calculated to be 38 kJ mol^{-1}. By making a comparison between activation enthalpy of (**3**) and that of rotation barrier about the central C–C bond of hexamethylethane ($\Delta H^{\ddagger}$= 22 kJ mol^{-1}),[6] the enthalpy originating from factor (*c*) was estimated to be −10 kJ mol^{-1}. The negative value of the enthalpy derived from factor (*c*) is explainable in terms of the conjugated enone system, C(14)=C(7)–C(8)=O, which is in a plane in the case of the transition state, while in the two ground states (two stable conformations), the enone systems were twisted with dihedral angles of −45.22 and 46.08°. Therefore the resonance stability was more effective in the transition state than in the ground states. Another conjugated enone system, C(10)=C(9)–C(8)=O, was negligible since it was almost planar in both the transition state and two ground states. The unusually high barrier to the conformational exchange process of (**1**) was caused by the hindered rotation about the single bond C(6)–C(11) and mainly by the non-bonded interaction between the two axial protons, 2-H and 4-H$_{ax}$, and one of the exomethylene protons, 14-H$_b$.

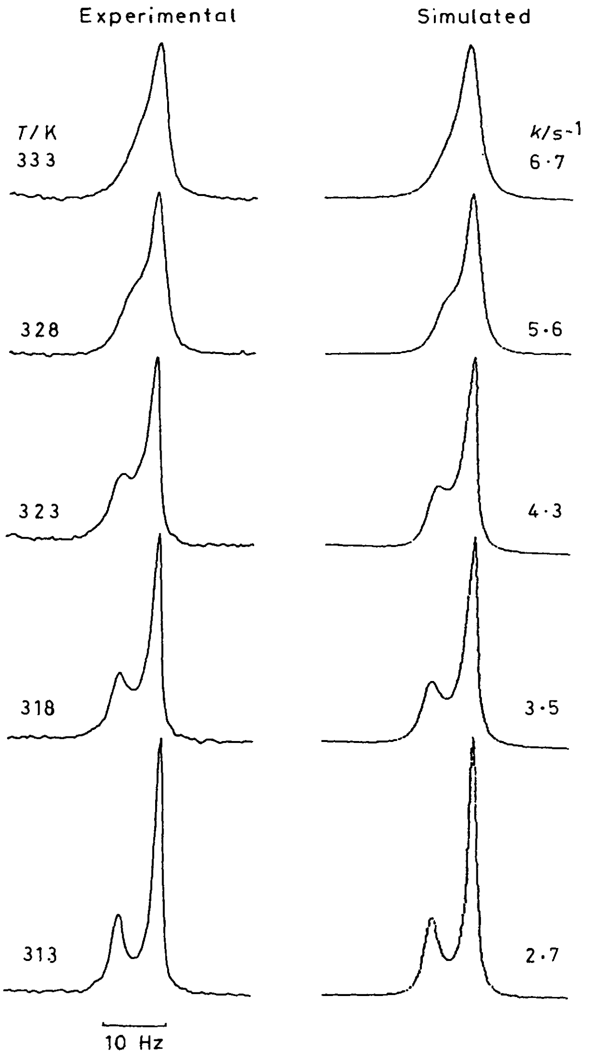


Figure 2. The experimental and simulated spectra of 14-H$_a$ of (**1**) as a function of temperature

The disparity between the stability of the two stable conformers could be explained in terms of the strain energy obtained by molecular-mechanics calculations. The dipole–dipole interaction energy was a major contributor to the difference in stability between the two conformers. The difference in the dipole–dipole interaction energy for (**1**) was estimated to be 1.69 kJ mol^{-1} (*ca.* 72% of the total strain difference). The other contributions to the stability difference were the bending energy and the torsional energy, each difference being 0.18 and 0.16 kJ mol^{-1}, respectively. In the case of (**2**), the inequality in the stability was mainly due to the dipole–dipole interaction energy, the difference of which was calculated to be 1.47 kJ mol^{-1} (*ca.* 53% of the total strain difference). The stretching and bending energies also rendered (**2a**) more stable than (**2b**). The difference in the stretching energy was 0.32 kJ mol^{-1} and that of the bending energy was 0.39 kJ mol^{-1}.

Experimental

The ^{1}H n.m.r. spectra were measured on a JEOL JNM-GX 500 or on a JEOL JNM-GX 270 spectrometer. The n.O.e.s were observed on a JEOL JNM-GX 270 spectrometer at 223 K. Dynamic n.m.r. spectra were recorded on a JEOL JNM-FX 100 spectrometer. All n.m.r. spectra were obtained in [$^2H_8$]toluene with $SiMe_4$ as an internal standard. Optical rotations were determined on a JASCO DIP-360 polarimeter. U.v. spectra were recorded on a Hitachi 200-10 spectrophotometer and i.r. spectra on a JASCO IR-S spectrophotometer. Mass spectra were taken at 70 eV with a JEOL JMS-DX 303 spectrometer. Spectral simulations were performed using the line-shape equation for a two-state model. Molecular-mechanics calculations were carried out at Hokkaido University Computing Centre.

2,10-*Dibromo*-3-*chlorochamigra*-7(14),9-*dien*-8-*one* (**1**).—(**1a**): δ(500 MHz; 298 K) 0.585 (2.1 H, s, 12-H_3), 0.765 (2.1 H, s, 13-H_3), 1.081 (0.7 H, ddd, *J* 15, 13, and 3.4 Hz, 5-H_{ax}), 1.155 (0.7 H, dddd, *J* 15, 4, 4, and 3 Hz, 5-H_{eq}), 1.407 (2.1 H, s, 15-H_3), 1.700 (0.7 H, ddd, *J* 14, 4, and 3.4 Hz, 4-H_{eq}), 1.776 (0.7 H, dd, *J* 14 and 13 Hz, 1-H_{ax}), 1.780 (0.7 H, ddd, *J* 14, 13, and 4 Hz, 4-H_{ax}), 2.117 (0.7 H, ddd, *J* 14, 5, and 3 Hz, 1-H_{eq}), 4.517 (0.7 H, dd, *J* 13 and 5 Hz, 2-H), 4.689 (0.7 H, s, 14-H_b), 5.819 (0.7 H, s, 14-H_a), and 6.294 (0.7 H, s, 9-H).

(**1b**): 0.598 (0.9 H, s, 13-H_3), 0.791 (0.9 H, s, 12-H_3), 1.039 (0.3 H, ddd, *J* 15, 14, and 5 Hz, 5-H_{ax}), 1.155 (0.3 H, dddd, *J* 15, 4, 4, and 3 Hz, 5-H_{eq}), 1.422 (0.9 H, s, 15-H_3), 1.802 (0.3 H, ddd, *J* 14, 5, and 4 Hz, 4-H_{eq}), 1.870 (0.3 H, dd, *J* 14 and 13 Hz, 1-H_{ax}), 2.059 (0.3 H, ddd, *J* 14, 14, and 4 Hz, 4-H_{ax}), 2.271 (0.3 H, ddd, *J* 14, 4, and 3 Hz, 1-H_{eq}), 4.185 (0.3 H, dd, *J* 13 and 4 Hz, 2-H), 4.683 (0.3 H, s, 14-H_b), 5.891 (0.3 H, s, 14-H_a), and 6.231 (0.3 H, s, 9-H).

Preparation of 10-*Bromochamigra*-2,7(14),9-*trien*-8-*one* (**3**).—To a suspended solution of pyridinium dichromate (1.0 mg) in CH_2Cl_2 (0.1 ml), was added a solution of 10-bromochamigra-2,7(14),9-trien-8-ol[1] (2.5 mg) in CH_2Cl_2 (0.1 ml). The mixture was stirred for 1 h at room temperature to yield (**3**) (1.4 mg) as a pale yellow oil; $[\alpha]_D^{22}$ $-$ 58.1° (*c* 0.17 in $CHCl_3$); $\lambda_{max.}$(EtOH) 260 (ε 7 800 dm^3 mol^{-1} cm^{-1}) and 202 nm (6 000); $\nu_{max.}$(film) 1 670, 1 600, 1 260, 1 030, and 882 cm^{-1}; δ(270 MHz; 300 K) 0.797 and 0.941 (each 3 H, s), 1.0–2.4 (6 H, m), 1.458 (3 H, s), 4.990 (1 H, s), 5.083 (1 H, br s), 6.147 (1 H, s), and 6.510 (1 H, s); *m*/*z* 296 and 294 (M^+, 28 and 29%), 281 and 279 ($M^+ - CH_3$, 24 and 23), 215 ($M^+ -$ Br, 39), 200 ($M^+ - CH_3 -$ Br, 30), and 41 (100).

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