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Studies on the Constituents of *Leptorumohra Migueliana* H. Ito. IV.[1] The NMR Studies of Protofarrerol and Triacetylfarrerol

Yukio Akahori, Seizi Katayama, Tadataka Noro, Seigo Fukushima, Yasuhisa Saiki, and Akira Ueno

Shizuoka College of Pharmacy[2]

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With the analysis of the nuclear magnetic resonance spectra of protofarrerol (IA) and triacetylfarrerol (IIC), the chemical shifts and the coupling constants containing the relative sign are determined using the double resonance techniques, the spin-decoupling and the spin-tickling. Especially the ABX spin systems in these compounds give the similar conformation and the same relative sign of coupling constants, even if the substituent of C-2 position is a phenyl or a alicyclic ring. The computations for the spin systems are carried out with the simulations and the iterations.

Introduction

In the previous papers, the isolation[3] and the determination of plane structure[4] of protofarrerol (IA) were reported, and its stereochemistry[1] was discussed. This paper describes the details of the structural studies using a high resolution nuclear magnetic resonance (NMR). The NMR spectra suggest that both IA and its derivative compound IIC[5] have the similar steric structure from the analysis of ABX spin system of C-2 and C-3 position. The comparison between IA and IIC is of special interest because of their structural uniqueness and their biological properties. Then there is the long range coupling between alicyclic C-6′ protons and C-2′ proton in IA. For the analysis of the various spin systems, the iterative method of the least square computation is used and the double resonance technique is applied

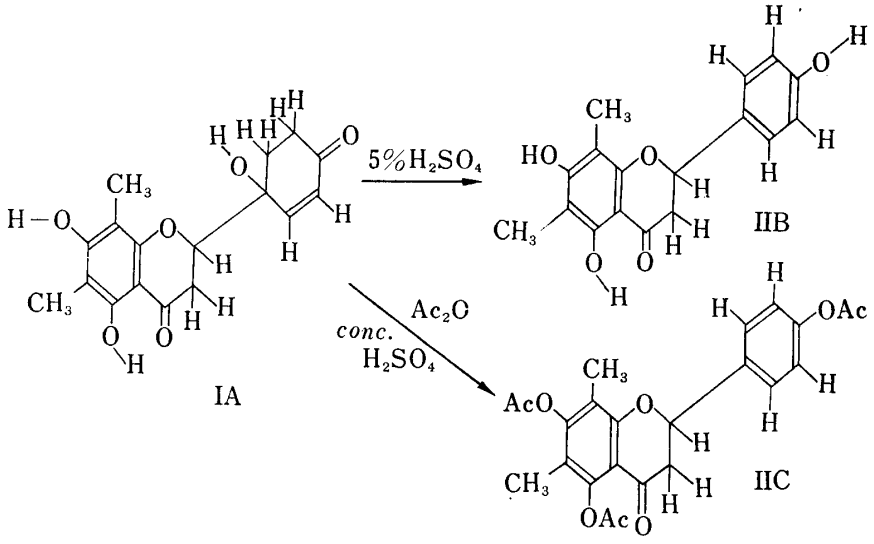

Chart 1

1) Part III: S. Fukushima, T. Noro, Y. Akahori, Y. Saiki, and A. Ueno, *Yakugaku Zasshi.*, **89**, 1272 (1969).
2) Location: *Ozika-160, Shizuoka.*
3) S. Fukushima, T. Noro, Y. Saiki, A. Ueno, and Y. Akahori, *Yakugaku Zasshi*, **88**, 1135 (1968).
4) T. Noro, S. Fukushima, Y. Saiki, A. Ueno, and Y. Akahori, *Yakugaku Zasshi.*, **89**, 851 (1969).
5) H. Arthur and Y. Kishimoto, *Chem. Ind.* (London), **1956**, 738.

for the experimental verification. The accurate values of the chemical shifts and coupling constants containing the relative sign are obtained. The relationship between these results and its steric structures is discussed in the present paper.

Experimental

Materials——Protofarrerol (IA) and Triacetylfarrerol (IIC) were prepared by the methods in the preceding papers. Solvents (C_5D_5N, $CDCl_3$, T.M.S.) were commercial products (spectra grade).

Recording of Spectra——The proton magnetic resonance spectra were obtained with a JNM-C-60-H high resolution NMR spectrometer (Japan Electron Optics Laboratory Co., Ltd.) and a JNM-SD-60 spin decoupler in case of spin-tickling[6] and spin-decoupling[7] experiments in solutions (IA: 10% w/v in C_5D_5N; IIC: 16% w/v in $CDCl_3$) containing T.M.S. as an internal reference at a room temperature (23°) operating the internal lock system and sweep rate 0.9 Hz/sec.

Preparation of Sample——The solutions of these samples were degassed by freezing and thawing several with times under high vacuum.

Analysis——The simulations and the iterative method of the least square for the analysis of complex NMR spectra are applied to a variety of the spin systems. The computation is carried out by NEAC-2200-model-200 and Fortran D-language, using the library program[8] of Tokyo University.

Results and Discussion

The 60 MHz NMR spectra of IA and IIC are shown in Fig. 1 and Fig. 2. The chemical shifts and the coupling constants of ABX spin systems are shown in Table I. These assignment are obtained by inspection of the spectra and are confirmed by the appropriate spin-decoupling experiments.

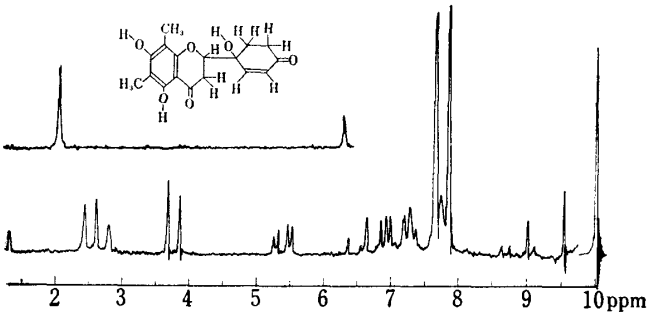

Fig. 1. A High Resolution Nuclear Magnetic Resonance Spectrum of Protofarrerol (IA)

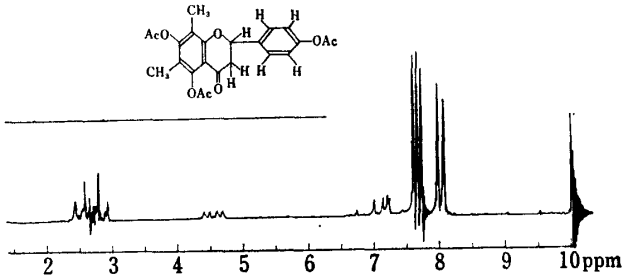

Fig. 2. A High Resolution Nuclear Magnetic Resonance Spectrum of Triacetylfarrerol (IIC)

Table I. The Chemical Shifts and the Spin–Spin Coupling Constants in ABX Spin Systems of Compound IA and Compound IIC (Hz)

	J_{AB}	J_{AX}	J_{BX}	δ_A	δ_B	δ_X
IA	−17.18	2.74	13.49	0.00	25.18	105.45
IIC	−16.58	2.56	13.69	0.00	12.71	159.53

The Analysis of NMR Spectrum of IA

The two sharp single peaks appeared in high-field region (τ 7.94, τ 7.75) are assigned to C-6 and C-8 methyl groups respectively, and the multiplet ($\tau \simeq 7.3$) arises from C-5′ methylene protons of the cyclohexenone ring and the multiplet ($\tau \simeq 7.7$) from C-6′ methylene protons, for the protons adjacent to carbonyl group generally appear in lower-field than the other.

6) R. Freemann and W.A. Anderson, *J. Chem. Phys.*, **37**, 2053 (1962).
7) J.D. Baldeschwieder and E.A. Randall, *Chem. Rev.*, **63**, 81 (1963).
8) No. 26, F2/TC/DIAG prepared by T. Shimizu.

So the AA'BB' spin system is composed of these four protons, but it is impossible to analyze these spin system accurately because of partly overlapping with the other lines.

The sharp doublet (τ 3.88) arises from C-3' olefine proton of cyclohexenone ring and the broaden doublet (τ 2.65) from C-2' olefine proton, since David and Woodgate[9] previously reported that the chemical shift of the olefine C-2' proton in cyclohexenone ring was situated lower field than that of C-3' position. The asymmetric AB spin system, therefore, is composed of the two protons. In this resonance spectrum, these significant broadening lines depend on the small coupling from the weak interaction to other protons. The strong irradiation on the broadening signal from the C-6' methylene protons removes the fine splittings from its protons, so that asymmetric AB pattern is replaced of symmetric AB (Fig. 3). The result indicates that there is the long-range coupling between C-2' and C-6' protons, and that its coupling corresponds to the "W" configuration[10] of the bonds.

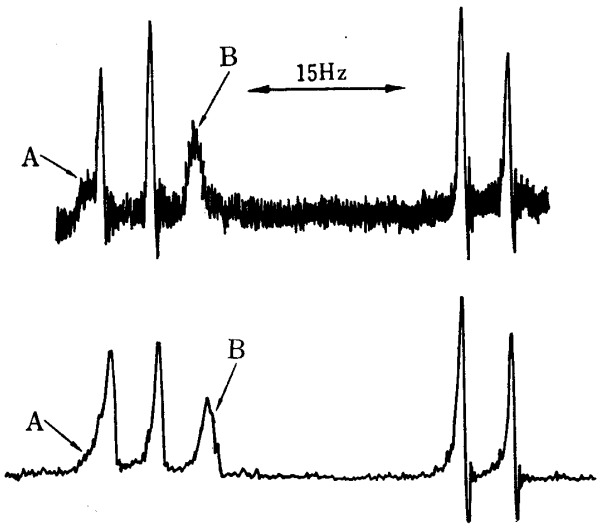

Fig. 3. The Upper Spectrum is the AB Spin System observed when a Second rf Field has been set on the C-6' Protons.

The lower is the original spectrum.
The peak A and B are arised from the solvent.

The C-2 proton (τ 5.5) in chromanone ring appears as quartet peak and the multiplet (τ 6.4$\simeq$7.4) arises from the C-3 two protons. The ABX spin system, therefor, is composed of only these three protons. The analysis of the ABX spin system are carried out accurately in following manners.

A high-speed computer is programmed to calculate a theoretical spectrum from a suggested set of parameter, and then to readjust these parameters to reproduce the experimental spectrum as well as possible. As the experimental data are in the form of transition frequencies and intensities, a major step in the analysis is to convert these into a set of energy levels and to order them correctly. This has remained strictly a trial and error method whether carried out by hand or by computer. Once the correct set of energy levels (eigen values of the spin Hamiltonian) has been established, the analysis is more straight-forward and it is usually possible to obtain the required parameters by iteration from an arbitrary set of starting parameter.

Improvement of these analysis procedures would be possible if additional experimental data could be obtained from the nuclear double resonance first suggested by Bloch.[11] In general, apart from the ambiguity in the sign of the spin coupling constant the transitions in a two-spin system may be assigned by inspection. This is not the case for three-spin system, but as noted by Freeman, *et al.* in their double-resonance studies it is possible to deduce not only the transitions but also the relative signs[12,13] of the spin coupling constants, *e.g.* when a appropriate line is irradiated with weak rf field H_2, connected lines having energy level in

9) B.P. David and P.D. Woodgate, *J. Chem. Soc.*, **1965**, 5943.
10) S. Sternhell, *Rev. Pure Appl. Chem.*, **14**, 15 (1964); E.W. Garbish, *Chem. Ind.* (London), **1964**, 1715.
11) F. Bloch, *Phys. Rev.*, **93**, 944 (1954).
12) A.D. Buckingham and J.A. Pople, *Trans. Faraday Soc.*, **1963**, 59.
13) H. Shimizu, *J. Chem. Phys.*, **40**, 3357 (1964).

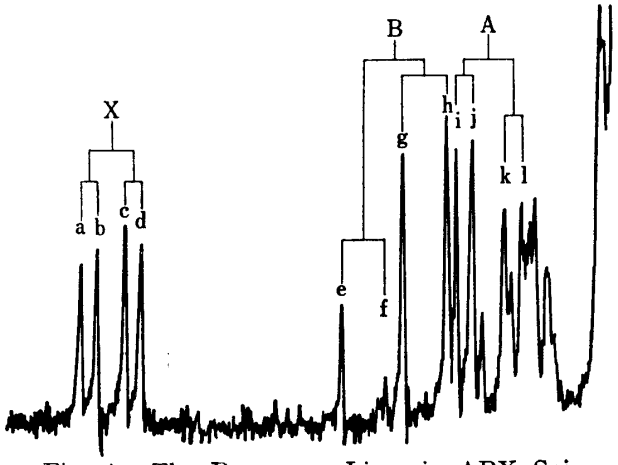

Fig. 4. The Resonance Lines in ABX Spin System are numbered a,b,.....,1 in Order of Increasing Frequency

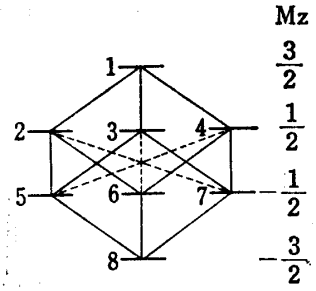

Fig. 5. The Energy-level Diagram of ABX Spin System in IA

common with the irradiated line may be observed to split into doublets. The perturbing effect of the second rf field used in such experiments depends upon the strength of this field (γ $H_2/2\pi$Hz).

When the each lines in the ABX spin system are numbered a, b, c, ··· etc in order of increasing frequency (Fig. 4), the energy-level diagram is as shown in Fig. 5 and the eight energy levels are numbered 1, 2, ···, 8. A second weak rf field are set on a resonance for a, and causes a splitting of lines for g and h as in practice show in Fig. 6-(B). Then analogous results holding for irradiation on lines b, c and d by the rf field H_2 is shown in Fig. 6(C), 6(D) and 6(E).

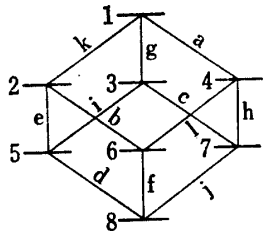

Fig. 7. The Correct Set of Transitions in IA. a, b, ···, k are the Resonance Lines

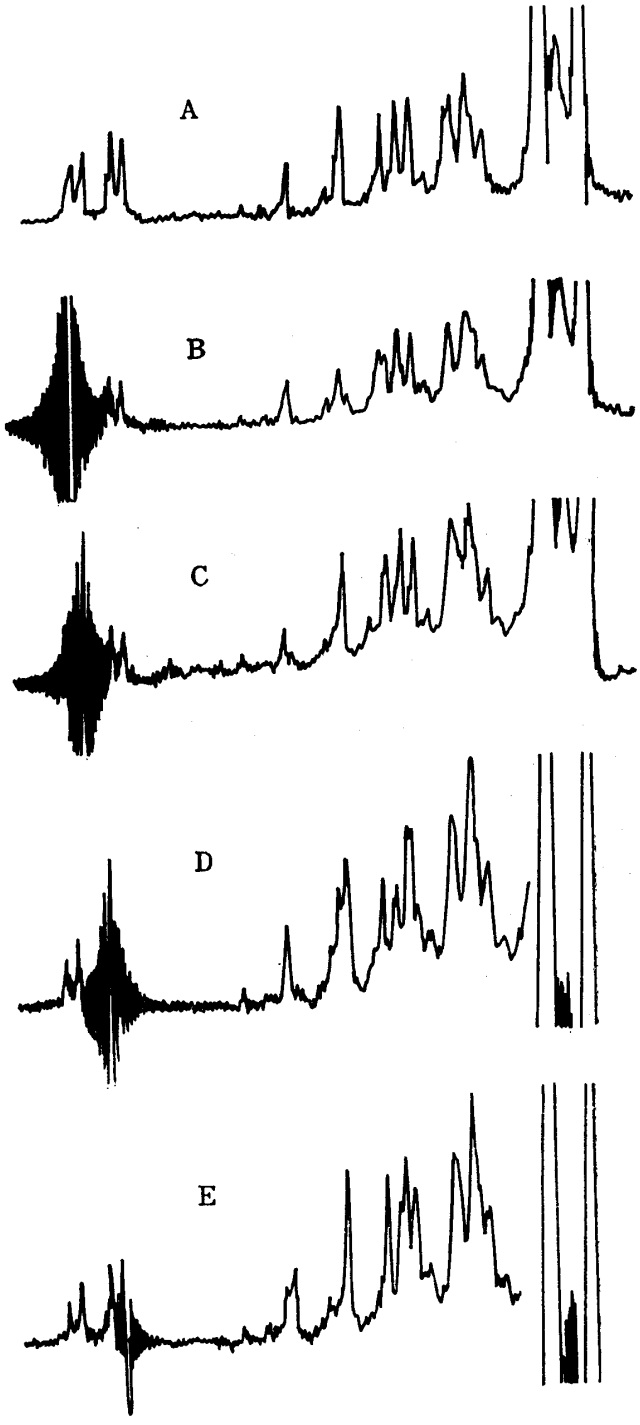

Fig. 6. The Spectrum A is the Original ABX Spin System in IA and the B is Observed Spectrum when a Weak rf field set on the Line a and C,D,E is observed Setting on the Line b,c,d respectively

Such the experimental spectrum corresponds to only one of the four possible arrangements of the spin-state table shown in Table II, that is, the relative sign of J_{AB}, J_{AX} and J_{BX} corresponding to −, + and + respectively. The correct set of transitions is established. These

Table II. The Spin State in ABX Spin System of Compound IA

	J_{AB}	J_{AX}	J_{BX}	a	b	c	d	e	f	g	h	i	j	k	l
i	+	+	+	4—1	6—2	7—3	8—5	3—1	7—4	5—2	8—6	2—1	6—4	5—3	8—7
ii	−	+	+	4—1	6—2	7—3	8—5	5—2	8—6	3—1	7—4	5—3	8—7	2—1	6—4
iii	+	−	+	6—2	4—1	8—5	7—3	3—1	7—4	5—2	8—6	6—4	2—1	8—7	5—3
vi	+	+	−	7—3	8—5	4—1	6—2	7—4	3—1	8—6	5—2	2—1	6—4	5—3	8—7

information is then used to construct the energy-level diagram which is represented in the form of a cube in Fig. 7. The chemical shift and the spin coupling constants obtained by iterative computation are $J_{AB}=-17.18$, $J_{BX}=2.74$, $J_{BX}=13.49$, $\delta_A=0.00$, $\delta_B=25.18$, $\delta_X=105.45$(Hz) The lines corresponding to transition (5—4),(6—3) and (7—2) are combination lines, and lines k and 1 can not be recognized because of the spectrum overlapping.

The Analysis of NMR Spectrum of IIc

The singlets (τ 8.07, τ 7.98) arise from the C-6 and the C-8 methyl protons, and the singlets (τ 7.74, τ 7.68, τ 7.62) are assigned to the methyl protons of acetyl groups. The C-3 methylene protons of chromanone ring appear as multiplet (τ 6.74—7.50) and the C-2 methine proton appears as quartet (τ 4.55). These three protons compose of ABX spin system. The identification of the transitions and the determination of the relative sign of the coupling constants in these ABX spin system are proved by the analogous manner (spin-tickling) as the case of IA. The weak rf field is set on the lines corresponding to proton A and B, the quartet from a X proton is observed as shown in Fig. 8.

When a line e is irradiated with a weak rf field, the connected lines b and d are observed to split into a doublet and the results of irradiation on the other lines are shown in detail in Fig. 8. The only case that is able to illustrate the relaitonship between such an experimental spectrum and the identification of the transitions in this spin system is one of taking the following relative sign of coupling constants; $J_{AB}=-$, $J_{AX}=+$, $J_{BX}=+$. Using these conclusions, the chemical shifts and the coupling constants are obtained by the iteration; $J_{AB}=-16.58$, $J_{AX}=2.56$, $J_{BX}=13.69$, $\delta_A=0.00$, $\delta_B=12.71$, $\delta_X=159.53$ (Hz).

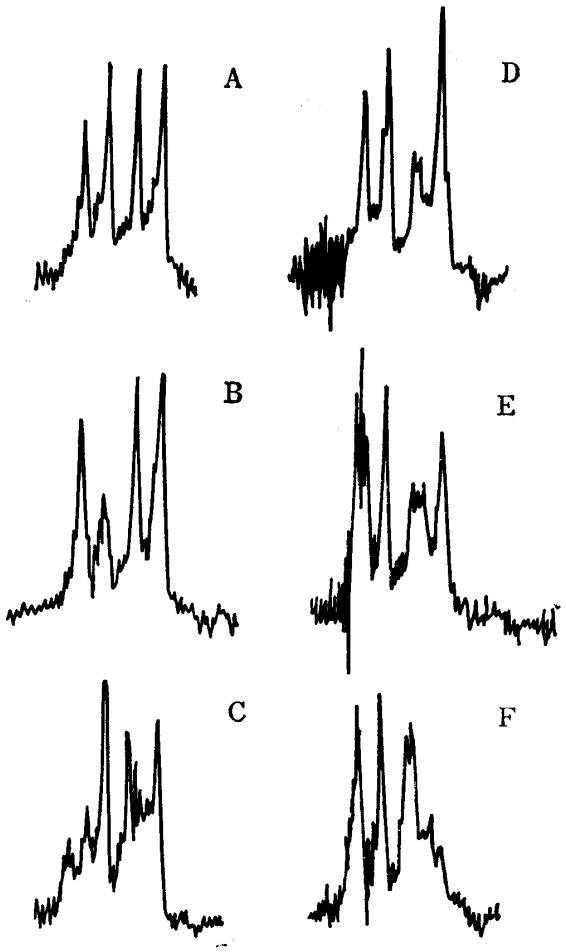

Fig. 8. The Spectrum A is the Original ABX Spin System in IIC and B is the Observed Spectrum when a Weak rf Field set on the Line e and C,D,E,F, are observed setting on the Line f,g, h, i, respectively

The multiplet (τ 2.4—3.0) arises from the four protons of phenyl group and indicates the AA'BB' spin system. Since these spectrum lines are poorly resolved, it is impossible to estimate by the iteration, and so their chemical shifts and the coupling constant is estimated by simulation; its values are J_{AB}

Table III. The Spin State in ABX Spin System of Compound IIC

	J_{AB}	J_{AX}	J_{BX}	a	b	c	d	e	f	g	h	i	j	k	l
i	+	+	+	4—1	6—2	7—3	8—5	3—1	7—4	5—2	2—1	8—6	6—4	5—3	8—7
ii	−	+	+	4—1	6—2	7—3	8—5	5—2	8—6	3—1	5—3	7—4	8—7	2—1	6—4
iii	+	−	+	6	4—1	8—5	7—3	3—1	7—4	5—2	8—6	6—4	2—1	8—7	5—3
vi	+	+	−	7—3	8—5	4—1	6—2	7—4	3—1	8—6	5—2	2—1	6—4	5—3	8—7

$=J_{A'B'}=8.7$, $J_{BB'}=2.5$, $J_{AA'}=2.3$, $J_{AB'}=J_{A'B}=0.4$ $\delta_A=\delta_{A'}=0.0$, $\delta_B=\delta_{B'}=9.6$ (Hz). The comparison between theoretical specturm and the original spectrum is shown in Fig. 9. The sharp singlets (τ 2.66, τ 2.74) are from impurities.

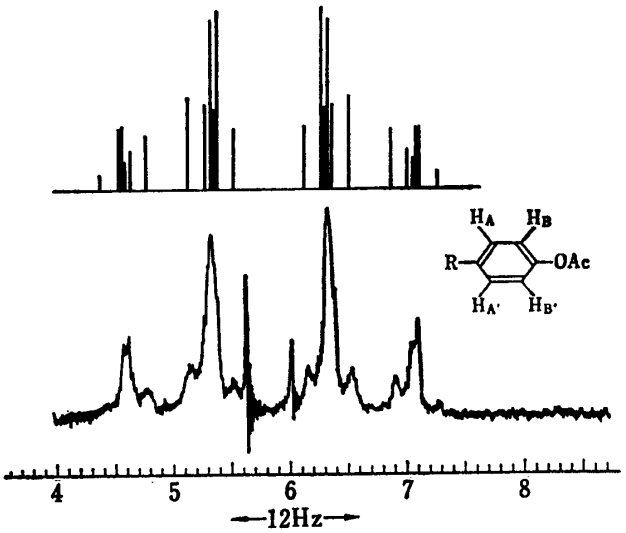

Fig. 9. The Upper Spectrum is the Theoretical Spectrum and the Lower Spectrum is the Original AA'BB' Spin System in IIC

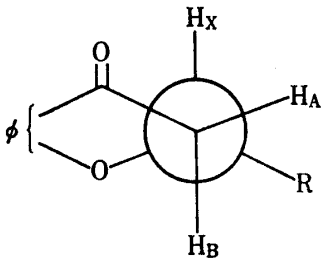

Fig. 10. The ABX Spin System in IA and IIC show a Similar Conformation

The ABX spin systems between IA and IIC indicate the exactly analogous conformation in Fig. 10. The dihedral angles of both compounds estimated from the Karplus[14] equation give the similar value, because of the magnitude of the observed coupling constants having the analogous values. It is interested that the spectrum lines corresponding to the transition (7—4) and the transition (5—3) of both compounds exchange its situation each other. From the results of these various NMR analysis described above, it is shown and comfirmed to be reasonable for the presumed structure of compound IA and IIC.

Acknowledgement Authors wish to thank the Shizuoka Computing Center and its personnel for help with the computation and for the use of library programs in part which were published by the Computing Center of Tokyo University.

14) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963); M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).