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The Application of Lanthanide Shift Reagent. I. Conformational Analysis of 1-[2-(1,3-Dimethyl-2-butenylidene)hydrazino]-phthalazine (DJ-1461) by Lanthanide Induced Shifts[1]

REIMEI MOROI, KEIICHI TOMITA, and MITSUJI SANO

Research Institute, Daiichi Seiyaku Co., Ltd.[2]

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The structure of DJ-1461 in solution was determined by using lanthanide shift reagents. The position of the lanthanide ion and the protons of DJ-1461 in the complex were determined using the McConnell-Robertson equation. The shifts induced by lanthanide shift reagents agreed well with the calculated values, on the assumption that their origin is of pseudocontact nature. The europium ion-N^2 bond length was as long as 3.2 Å (phthalazine N^2 is set at the origin of the right handed polar co-ordinates system). It was found that the side chain of the DJ-1461 has an *anti* form towards the C=N bond, and the two double bonds S-*cis* configuration.

The lanthanide-induced-shift method has drawn much attention since its discovery in 1969.[3] Simplification of proton magnetic resonance (PMR) spectra by the addition of lanthanide shift reagent (LSR) provides a convenient technique for the qualitative analysis of spectra of compounds. If the LSR is used quantitatively to deal with the data, it would be possible to determine the molecular conformation as in solution.[4]

DJ-1461 which has an antihypertensive effect is made of a rigid frame and a flexible side chain. The lanthanide-induced-shift method could be successfully used for the analysis of molecular conformation of DJ-1461 in solubilized form.

The lanthanide–substrate interaction is mainly of pseudocontact nature expressed by the McConnell–Robertson equation.[5]

$$Hi/H = K\frac{3\cos^2\theta i-1}{Ri^3} \qquad (1)$$

Where Hi is the induced shift of ith proton. θi is the angle made by this vector and the principle molecular axis of the complex. Ri is the distance between the metal and the ith proton, K being nearly a constant and may be assumed to be a scale factor for a given experimental set. This equation had been discussed about its pseudocontact terms, whether it is true or not.[6] But the pseudocontact terms have been accepted as a true one.[7]

This paper deals with FORTRAN programs in order to simulate a molecular conformation and describs the result of structural study of DJ-1461.

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1) This work was presented at the 8th Congress of Heterocyclic Chemistry, Kyoto, October 1975.
2) Location: *Minamifunabori-cho, Edogawaku, Tokyo.*
3) C.C. Hinckley, *J. Am. Chem. Soc.*, **91**, 5160 (1969).
4) C.D. Barry, A.C.T. North, J.A. Glasel, R.J.P. Williams, and A.V. Xavier, *Nature*, **232**, 236 (1971).
5) H.M. McConnell and R.E. Robertson, *J. Chem. Phys.*, **29**, 1361 (1958).
6) *a*) B. Bleaney, C.M. Dobson, B.A. Levine, R.B. Martin, J.P. Williams, and A.V. Xavier, *Chem. Commun.*, **1972**, 791; *b*) R.M. Wing, J.J. Uebel, and K.K. Andersen, *J. Am. Chem. Soc.*, **95**, 6046 (1973).
7) *a*) D.R. Eaton, *J. Am. Chem. Soc.*, **87**, 3097 (1965); *b*) R. von Ammon and R.D. Fischer, *Angew. Chem., Intern. Ed. Engl.*, **11**, 675 (1972); *c*) K. Tori, Y. Yoshimura, M. Kainosho, and K. Ajisaka, *Tetrahedron Letters*, **1973**, 1573; *d*) J.D. Roberts, G.E. Hawkes, J. Husar, A.W. Roberts, and D.W. Roberts, *Tetrahedron*, **30**, 1833 (1974).

Experimental

Materials——DJ-1461 was synthesized according to the method of K. Ueno, *et al.*[8] The lanthanide shift reagents [$Eu(DPM)_3$, $Pr(DPM)_3$ and $Gd(DPM)_3$][9] were purchased from Willow Brook Laboratories, Inc (Waukesha, Wisconsin).

Lanthanide Induced Shift Measurement——Samples for shift measurement made up to contain DJ-1461 at the concentration of 6.25 mM in 0.5 ml of deuterochloroform containing 0.3% tetramethylsilane (TMS). The LSR were added in the range of 0.1 to 1.5 mM upon DJ-1461. PMR measurements were made by a Hitachi R-20B spectrometer (60 MHz) in the frequency-swept mode at a probe temperature of 35°. Chemical shifts were reported in parts per million from TMS.

Fluorescence spectra of the compound were obtained in CCl_4 solution on a Hitachi MPF-2A spectrophotometer.

The computation were carried out on a JEOL JEC-6 spectrum computer.

Results and Discussion

Computation

Program consist of the two parts named LP and FRP. The LP is used for the determination of lanthanide position and the FRP is used for the determination of the position of free rotational proton.

A molecular conformation is determined using the LP and FRP. A molecular was expressed with respect to the polar coordinates system. Figure 1 shows the relation between the polar coordinates and rectangular coordinates. The position of lanthanide ion (L) is expressed dÅ (the assumed L-Lewis base distance), ρ (the colatitude, measured from the positive Z axis) and ϕ (the azimuth, measured counterclockwise from the X-Z plane). Hi (ith proton) is similarly expressed by d'Å (Hi-Lewis base distance), ρ' (the colatitude) and ϕ' (the azimuth). When the L and Hi are defined as mentioned above, the following equation is obtained.

$$x = d\cos\phi\sin\rho, \quad y = d\sin\rho\sin\phi, \quad z = d\cos\rho.$$

$$r = [(x-xi)^2+(y-yi)^2+(z-zi)^2]^{1/2}$$

$$\cos\theta i = \frac{r^2+d^2+d'^2}{2rd}$$

Therefore, if the coordinates of the lanthanide ion is chosen by combining the d, ρ and ϕ at will, the various calculated shifts data can be obtained by applying the equation (1) and can be compared with observed shifts. The difference between observed and calculated shifts values for a given set of d, ρ and ϕ values is expressed as an agreement factor (R).[10] The minimum value of the R factor indicates a lanthanide position.

$$R = \left[\frac{\sum_i (H_{oi}-H_{ci})^2}{\sum_i (H_{oi})^2}\right]^{1/2} \qquad \begin{array}{l} H_{oi}\text{: observed shift} \\ H_{ci}\text{: calculated shift}\end{array}$$

In calculating a position of free rotational proton of the compound, we change the polar coodinates axis so that the locus of the rotamor can be obtained as shown in Fig. 2. All the positions on the locus (the angular search increment for each rotation is 1°) are calculated in order to have the position, which give much smaller value of the R factor. Thus, the position is moved slightly in a small space in order to have the minimum value of the R factor which give the best position. When the FRP is running, the position of the lanthanide ion is fixed. If a compound has a flexible chain, the analysis is started on the basis of part of the rigid frame.

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[8] K. Ueno, R. Moroi, M. Kitagawa, K. Asano, and M. Miyazaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 1068 (1976).

[9] Abbreviations used: $Eu(DPM)_3$ for tris (dipivalomethanato) europium(III); Pr $(DPM)_3$ for tris (dipivalomethanato)praseodium(III); $Gd(DPM)_3$ for tris (dipivalomethanato)gadolinium(III).

[10] *a*) M.R. Willcott III, R.E. Lenkinski, and R.E. Davis, *J. Am. Chem. Soc.*, **94**, 1742 (1972); *b*) R.E. Davis and M.R. Willcott, III, *ibid.*, **94**, 1744 (1972).

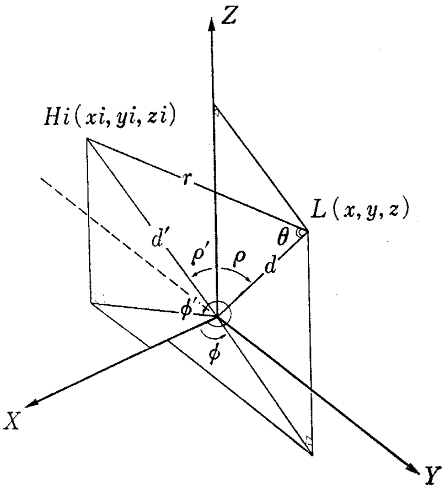

Fig. 1. The Co-ordinates System

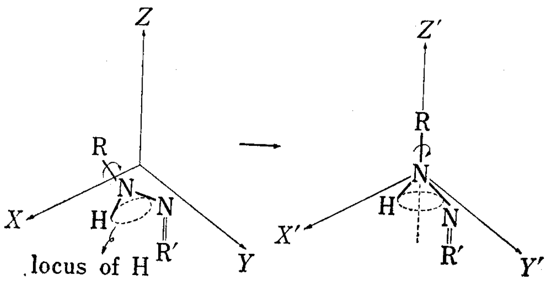

Fig. 2. The Transformation of Co-ordinates.

The lanthanide position is determined using the LP, to begin with. Next the position of flexible proton is determined using the FRP. The computer input consist of values of the coordinates of the rigid frame (d', ρ' and ϕ') consistent with the PMR data.

FORTRAN Program

The LP and FRP were written with the JIS 3000. Fig. 3 and 4 show the flow charts of LP and FRP.

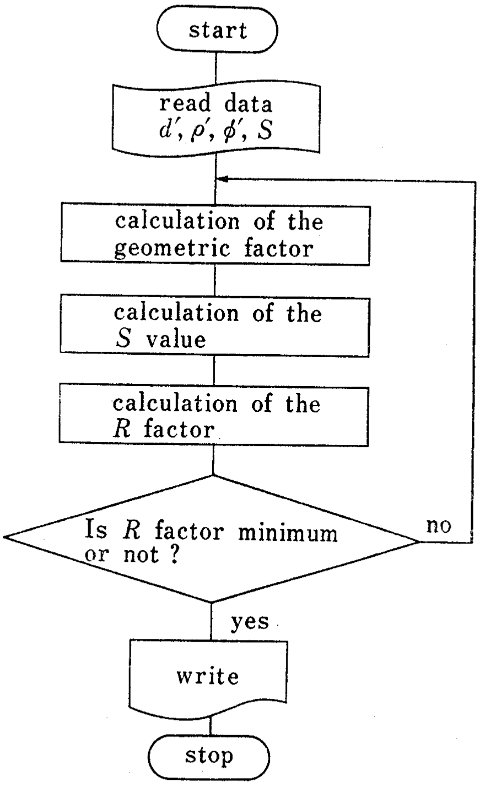

Fig. 3. Flow Diagram of the LP Program

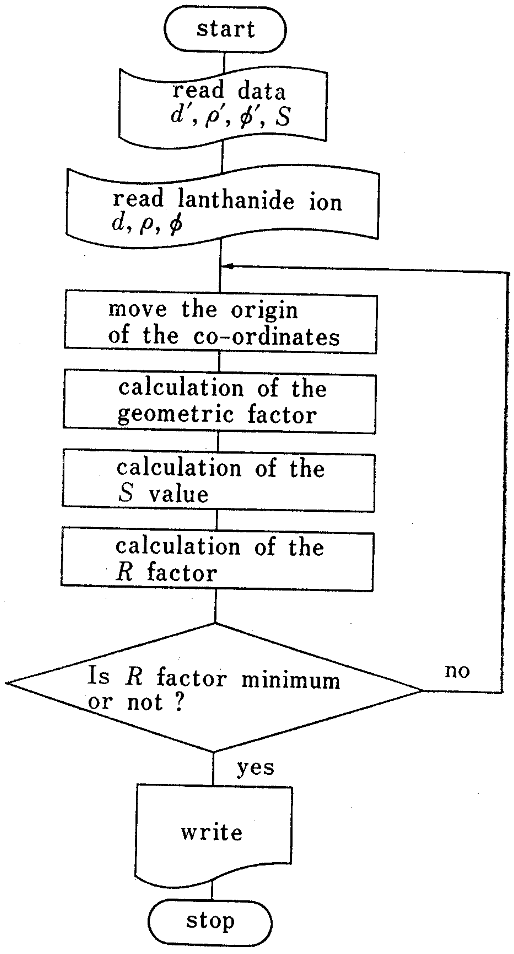

Fig. 4. Flow Diagram of the FRP Program

Assignment of the Chemical Shifts

The PMR spectrum of DJ-1461 showed absorptions corresponding to three methyl groups (1.92, 2.10 and 2.30 ppm), one olefinic proton (5.92 ppm), five aromatic protons (7.30—7.72, 8.20—8.60 ppm), one NH proton (10.30 ppm) as shown in Fig. 5. Signals of d and e methyl groups and signals of 5-H and 8-H were assigned as follows; The nuclear Overhauser effect (N-OE) were observed in e methyl group (Table I) and in 8-H signal (Table II). The NOE was measured in the presence of Eu $(DPM)_3$. The integral peak area of 4-H signal was much greater when 5-H proton was irradiated than 8-H proton. The assignment of the chemical shifts was summarized in Table III.

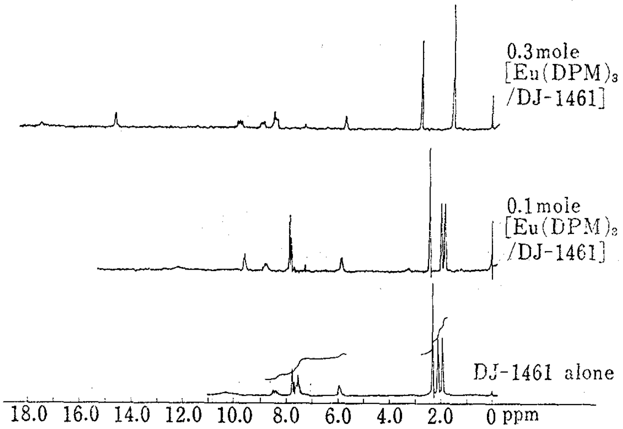

Fig. 5. Spectra of DJ-1461 in $CDCl_3$ in Absence and in Presence of Eu $(DPM)_3$

Table I. Nuclear Overhauser Effects

Irradiation	Area of the signal c-H	Increase in %
none	6.21	
at e-Me	6.46	4.03
at d-Me	6.20	0

These data are average of seven different runs.

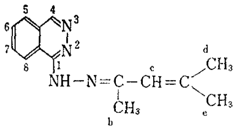

Table II. Nuclear Overhauser Effects

Irradiation	Area of the signals 5-H	Area of the signals 8-H	Increase in %
None	14.07	13.51	−0.4(5-H)
at b-Me	14.02	14.28	5.7(8-H)

These data are average of ten differerent runs.

Table III. PMR Spectral Data of DJ-1461

Position	Chemical shift	Position	Chemical shift
4-H	7.72	NH	10.30
5-H	7.30—7.72(9.85)$^{a)}$	b-Me	2.30
6-H	7.30—7.72(8.98)$^{a)}$	c-H	5.92
7-H	7.30—7.72(8.98)$^{a)}$	d-Me	1.92
8-H	8.37(10.77)$^{a)}$	e-Me	2.10

a) Chemical shifts are reported when 0.4 mM of $Eu(DPM)_3$ were added for 1.0 mM of DJ-1461.

Behavior of Induced Shift

Fig. 6 and 7 show the $Eu(DPM)_3$- and $Pr(DPM)_3$-induced shifts of DJ-1461. An approximately linear relationship was observed between the induced shifts and the molar ratio of shift reagents. The ratio of shifts induced by $Eu(DPM)_3/Pr(DPM)_3$ was nearly 1 except for e-Me as shown in Table IV. This ratio indicated that the induced shifts were of pseudocontact nature in origin.

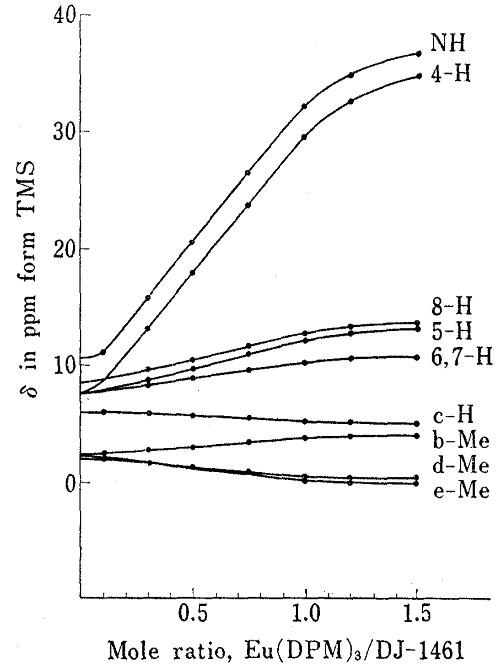

Fig. 6. Induced Chemical Shifts Data for DJ-1461 with Eu(DPM)$_3$

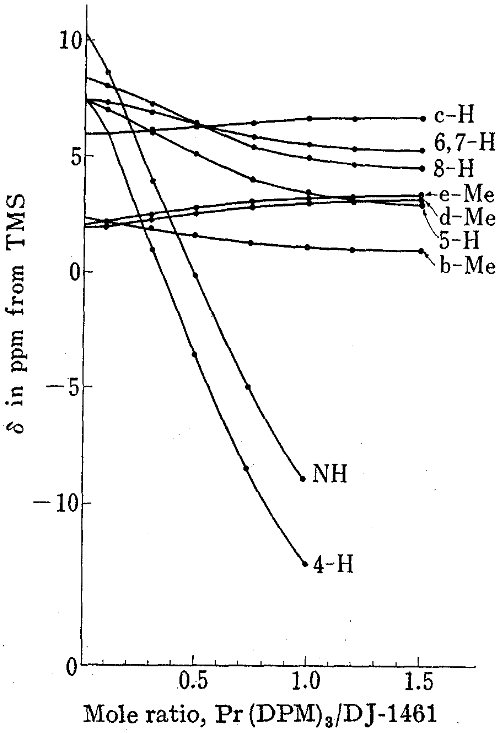

Fig. 7. Induced Chemical Shifts Data for DJ-1461 with Pr(DPM)$_3$

Table IV. Induced Chemical Shift Data for DJ-1461

Proton	S$^{a)}$Eu (DPM)$_3$	S$^{a)}$Pr (DPM)$_3$	S$^{a)}$Eu (DPM)$_3$/S$^{a)}$Pr (DPM)$_3$
4-H	21.90	−23.48	1.07
5-H	4.57	−4.84	1.06
6-H	2.56	−2.38	0.93
7-H	2.56	−2.38	0.93
8-H	4.32	−4.08	0.94
NH	22.00	−21.86	0.99
b-Me	1.37	−1.39	1.01
c-H	−0.77	0.68	0.88
d-Me	−1.39	1.24	0.89
e-Me	−1.96	1.29	0.66

a) S expressed as (δ value in ppm for 1:1 mole ratio of Ln(DPM)$_3$: DJ-1461) minus (δ value for extrapolating point back to zero mole of Ln(DPM)$_3$). Minus indicate upfield shift.

The Gd(DPM)$_3$ is reported its effectiveness in the check of the contribution of contact shift.[11] When this reagent was used in a small quantity for DJ-1461, there were no changes for chemical shifts. With increasing amount of the reagent, the broadening of the spectrum became intensified. Therefore the induced shifts could be deduced as pseudocontact nature in origin.

In order to clarify the stoichiometry of the complex in solution, fluorescence spectrum was measured for Pr(DPM)$_3$-DJ-1461 complex. The results showed that the ralative intensity of the complex became constant when the mole ratio [Pr(DPM)$_3$/DJ-1461] reached to 1 (Fig. 8). This indicated that the complex was consisted of 1 mole of Pr(DPM)$_3$ and 1 mole of DJ-1461.

Calculation for Flexible Chain when Eu(DPM)$_3$ was used

The computer input consisted of values of *d′*, *ρ′*, *ϕ′* and chemical shifts (*S* value).

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[11] W.D. Horrocks, Jr., and J.P. Sipe, III., *J. Am. Chem. Soc.*, 93, 6800 (1971).

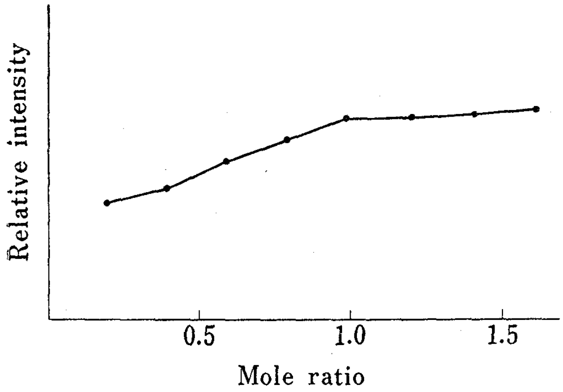

Fig. 8. The Dependence of Fluorescence Intensity of the Complex upon Mole Ratio [Pr(DPM)$_3$/DJ-1461]

The fluorescence intensity was measured at 362 nm (excitation)and 465 nm (emission).

Table V. Polar Co-ordinates of Rigid Part in DJ-1461

Proton	d'(Å)	ρ'(°)	ϕ'(°)
4–H	3.32	21.0	0
5–H	4.83	50.3	0
6–H	6.10	71.4	0
7–H	5.82	94.4	0
8–H	4.07	114.6	0

Phthalazine ring is set at the X–Z plane and the phthalazine N^2 is set at the origin. Bond direction of N^2→N^3 is set at the positive Z axis.

The analysis was started on the basis of part of the rigid frame (phthalazine ring). The position of europium ion (Eu) was first searched with LP by the use of the coordinates of the phthalazine ring. The Eu was searched over the 1.8 to 5.5 Å at 0.1 or 0.2 Å intervals, and the angles ρ and ϕ were scanned over all ranges at 1° to 10° intervals. The R factor was used for a guide of calculation.

Table V shows the coordinates of the phthalazine ring expressed by the polar coordinates which were measured from Büch Dreiding model.

Position of Eu——The position of Eu was obtained as d=2.3 Å, ρ=30° and ϕ=320°. These values were determined by the use of the coordinates of the phthalazine ring only. Therefore the values were thought to be changeable.

Position of NH——In order to obtain the coordinates of the NH, the FRP was run using the position of Eu. As a result, the coordinates of the NH was d'=2.3 Å, ρ'=176.6° and ϕ'=353.0°. Again the LP was run for searching a new position of Eu by adding the coordinates of the NH. The result was d=2.6 Å, ρ=115°, and ϕ=121°. These alternate calculations between the LP and FRP were performed till the position of Eu was convergent. The calculation process was shown in Table VI. The position of Eu was d=3.2 Å, ρ=95°, and ϕ=279°. The position of NH was d'=3.1 Å, ρ'=139.1°, and ϕ'=4.7°. Figure 9 shows the plot of R factor at NH as a funcion of ϕ', where d' and ρ' were fixed at 3.1 Å and 139.1°, respectively.

Table VI. Polar Co-ordinates of NH

Program	R %	Lanthanide ion			NH		
		d(Å)	ρ(°)	ϕ(°)	d'(Å)	ρ'(°)	ϕ'(°)
LP	2.37	2.3	30	320			
FRP	20.13	2.3	30	320	2.3	176.7	353.0
LP	11.09	2.6	115	121			
FRP	10.97	2.6	115	121	2.8	145.6	322.6
LP	3.99	3.3	94	282			
FRP	3.99	3.3	94	282	3.1	139.1	4.7
LP	3.99	3.3	94	282			
FRP	4.03	3.2	95	279	3.1	139.1	4.7

Position of b-Me, c-H, d-Me, and e-Me——After analysis of all protons or methyl groups, the values given in Table VII were obtained. The position of methyl group was described with a center of circle which was drawn by three protons. The shift ratio of e-Me was 0.66. So

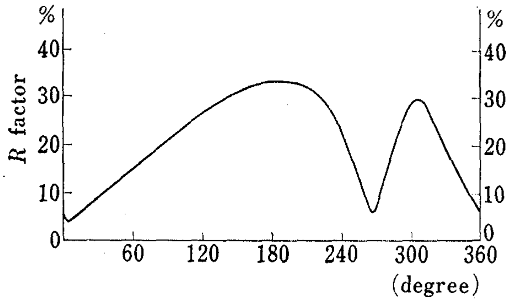

Fig. 9. Variation of R Factor for NH

Table VII. Polar Co-ordinates of Flexible Part

Proton	d (Å)	ρ(°)	ϕ(°)
Eu ion	3.2	95	279
NH	3.1	139.1	4.7
b-Me	5.2	156.6	1.0
c-H	5.8	164.7	215.2
d-Me	3.5	123.5	210.0
e-Me	6.0	132.6	203.3

Table VIII. Comparison of Observed and Calculated $Eu(DPM)_3$-Induced Shifts

Lanthanide position $d=3.2$Å $\rho=95°$ $\phi=279°$ $R=6.59\%$	Observed	Calculated
4-H	21.90	21.82
5-H	4.57	4.92
6-H	2.56	2.40
7-H	2.56	0.97
8-H	4.32	3.58
NH	22.00	22.25
b-Me	1.37	1.70
c-H	−0.77	−0.24
d-Me	−1.39	−1.04
e-Me	−1.96	−1.16

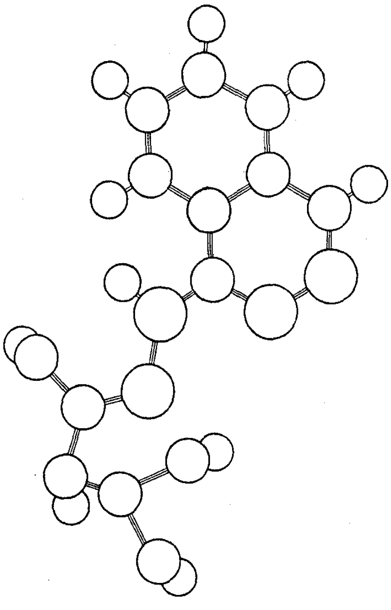

Fig. 10. ORTEP[12) Drawing of the Molecular Structure of DJ-1461 Viewed along the Y Axis

the induced shift of e-Me was not always of pseudocontact nature. Nevertheless we attempted the calculation of the coordinates. Table VIII shows the observed and calculated shifts.

The molecular structure of DJ-1461 was determined by a series of calculations mentioned above. The structure shown in Fig. 10 is in the presence of $Eu(DPM)_3$ in solution. The NH and b-Me were nearly in the same plane with the phthalazine ring. The side chain of DJ-1461 has an *anti* form towards the C=N bond, and the two double bonds S-*cis* configuration.

There are four nitrogen atoms in DJ-1461. The nearest nitrogen to the Eu was at the 2-position of the phthalazine ring (N^2; 3.20 Å, N^3; 3.63 Å, NH; 3.64 Å, C=N; 4.20 Å). So the Eu was unexpectedly coordinated with DJ-1461 at the 2-position of the phthalazine ring. However the position of Eu was thought to be the least steric hinderside. The phthalazine ring and the side chain in DJ-1461 are roughly on the same plane. If the LSR were to be coordinated

Table IX. Variation of R Factor

Variation of the bond length ($Eu-N^2$)			R%
$d=3.4$Å	$\rho=95$	$\phi=279$	17.56
$d=3.3$Å	$\rho=95$	$\phi=279$	11.04
$d=3.2$Å	$\rho=95$	$\phi=279$	6.59
$d=3.1$Å	$\rho=95$	$\phi=279$	10.54
$d=3.0$Å	$\rho=95$	$\phi=279$	18.85

Table X. Comparison of Observed and Calculated Pr(DPM)$_3$-Induced Shifts

Lanthanide position d=3.1Å ρ=95° ϕ=280° R=8.36%	Observed	Calculated
4-H	−23.48	−22.93
5-H	−4.84	−5.18
6-H	−2.38	−3.03
7-H	−2.38	−1.33
8-H	−4.08	−2.52
NH	−21.86	−22.35
b-Me	−1.39	−2.15
c-H	0.68	−0.77
d-Me	1.24	1.74

with DJ-1461 from the side direction (nitrogen side) of the plane of the phthalazine ring, there would be a large steric hindrance between the pivaloyl groups of LSR and three methyl groups of the side chain. This complex would be unstable. In this case the value of R factor became extremely poor, *i.e*, R=36.07%, assumed Eu position d=3.2 Å, ρ=95°, and ϕ=180°. DJ-1461 is changeable to its geometric isomer in the presence of HCl or BF$_3$.[8] This indicated that the position of hydrochloride or boron trifluoride of DJ-1461 was at the nitrogen atom of the C=N bond of the side chain. The lanthanide shift reagent, though it is Lewis acid, did not isomerize DJ-1461. This indicated that the position of Eu was different from that of the hydrochloride or boron trifluoride.

When the position of Eu was searched by the use of a part of the data, it was different from final one. This suggested that the minimum R factor led to not always the correct position, and the R factor would give a favorable result when whole data were used for calculation.

The changes of the Eu–N^2 distance affected to the R factor as shown in Table IX. This is not coincident with results of Willcotts III, *et al.*[10a]

In case of Pr(DPM)$_3$, the position of praseodium ion was d=3.1 Å, ρ=95°, and ϕ=280° (Table X). The Pr–N^2 distance was shorter than the Eu–N^2 distance by 0.1 Å.

Acknowledgement The authors wish to express their deep gratitude to Dr. G. Ohta, Director of this Institute and Dr. K. Ueno, for their interest and encouragement.

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12) C.K. Johnson, ORTEP. Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1965. When we applied this program, all the parameters were treated as a constant.