where J_θ is as given in eq 1. The $J_{\alpha CH-\beta CH}$ for structure I is 13 Hz with no significant temperature dependence, whereas for structure IV, a value of 5 Hz is obtained at 25°. The experimental value is 11 Hz for the valyl residues of the valinomycin-K^+ complex with no change in temperature over the range of -50 to 30°. The experimental result for the side chain of hydroxyisovaleric acid is 2.5 Hz at $-50°$ and 4 Hz at 30°. This result quite conclusively defines the solution conformation of the valinomycin-K^+ complex to be conformer I.

This result is the same as that reported from the crystal studies for the backbone atoms.[20] The X-ray coordinates have not yet been reported for the backbone atoms nor have positions of the side chains been reported. It will be of interest when the X-ray coordinates are reported to compare them with those given in Table I.

(20) M. Pinkerton, L. K. Steinrauf, and P. Dawkins, *Biochem. Biophys. Res. Commun.*, 35, 512 (1969).

Crystal Structure and Conformation of the Cyclic Dipeptide cyclo-L-Prolyl-L-leucyl

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Contribution from the Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D. C. 20390. Received June 9, 1971

Abstract: A single-crystal X-ray diffraction analysis has been made of the structure of the cyclic dipeptide cyclo-L-Pro-L-Leu. The diketopiperazine ring has a folded conformation with a dihedral angle of 143° between the two planar peptide units. In the pyrrolidine ring, C^β is 0.52 Å out of the plane of the other four atoms. The leucyl side chain is fully extended. Molecules form infinite chains parallel to the a axis by means of NH$\cdots$O hydrogen bonds. The material crystallizes in the orthorhombic space group $P2_12_12_1$ with cell parameters $a = 9.451$, $b = 19.587$, and $c = 6.340$ Å. The X-ray intensity data were collected with an automatic diffractometer and refined to $R = 6.4\%$. The crystal structure was solved by the symbolic-addition procedure for noncentrosymmetric crystals.

Cyclic dipeptides contain the diketopiperazine (DKP) ring which in the crystalline state has been found to be planar when unsubstituted, *i.e.*, in cyclo-Gly-Gly[1] (I), and nearly planar in cyclo-D-Ala-L-Ala[2] (II).
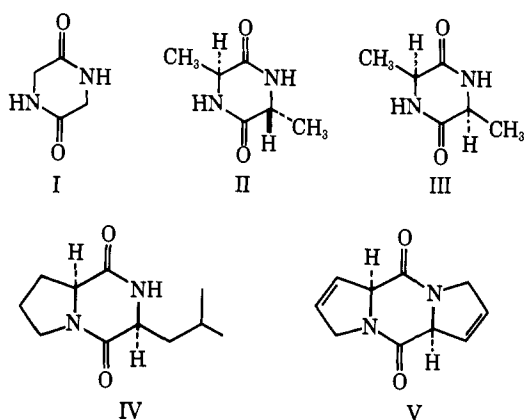
Both of these molecules possess a center of symmetry. In cyclo-L-Ala-L-Ala[2] (III), on the other hand, the DKP ring is appreciably puckered and has been described as a twist boat. In the present investigation of cyclo-L-Pro-L-Leu (IV), the side groups on the DKP ring are dissimilar and bulkier than those previously studied. In this case, the ring has been found to have a symmetric

boat shape, with all parameters almost identical with those found in 3,4-dehydroproline anhydride[3] (V).

In addition to the conformation of the DKP ring, the conformation of the pyrrolidine ring is of interest. In L-proline and other pyrrolidine-related amino acids, the pyrrolidine ring has assumed a number of conformations in the crystalline state as shown in Table I. The five-membered ring has the envelope conformation, with four atoms in a plane and one out of the plane by 0.4–0.6 Å either on the same side as or on the opposite side to the carboxyl group. In the various prolines, atom C^β, C^γ, or N is out of the plane. In the prolyl residues in the linear polypeptides tosyl-L-Pro-L-Pro(OH),[4] L-Leu-L-Pro-Gly,[5] and p-bromocarboxy-Gly-L-Pro-L-Leu-Gly(OH),[6] atom C^γ is 0.26–0.60 Å out of the plane of the other four atoms. However, in cyclo-L-Pro-L-Leu, it is C^β which is 0.52 Å out of the plane.

Experimental Section

A large colorless crystal of cyclo-L-Pro-L-Leu, in the shape of a hexagonal plate, was grown by slow evaporation from ethyl acetate solution by Dr. T. C. McMorris of the New York Botanical Garden. The soft crystal was cut to a more suitable shape, with dimensions 0.3 × 0.5 × 1.0 mm, for the diffraction experiment. X-Ray intensities were measured with copper radiation on a four-circle automatic diffractometer using the θ-2θ scan technique with a 2.4° +

(1) R. Degeilh and R. E. Marsh, *Acta Crystallogr.*, 12, 1007 (1959).
(2) (a) E. Sletten, *J. Amer. Chem. Soc.*, 92, 172 (1970); (b) E. Benedetti, P. Corradini, and C. Pedone, *Biopolymers*, 10, 221 (1971); (c) *J. Phys. Chem.*, 73, 2891 (1969).

(3) I. L. Karle, manuscript in preparation.
(4) J. Fridrichsons and A. McL. Mathieson, *Acta Crystallogr.*, 15, 569 (1962).
(5) Y. C. Leung and R. E. Marsh, *ibid.*, 11, 17 (1958).
(6) T. Ueki, T. Ashida, M. Kakudo, Y. Sasada, and Y. Katsube, *ibid.*, B, 25, 1840 (1969).

Table I. Conformations of the Pyrrolidine Ring in
Natural Prolines

Conformation	Dev from least-squares plane, Å	Ref
	C^γ 0.6 cis	a
	C^γ 0.4 trans	b
	C 0.6 cis	c
	N 0.4 cis	d
	N 0.4 trans	d

 a R. L. Kayushina and B. K. Vainstein, *Sov. Phys.-Crystallogr.*,
10, 698 (1966). *b* J. Donohue and K. N. Trueblood, *Acta Crystal-
logr.*, **5**, 414, 419 (1952). *c* I. L. Karle, *ibid.*, *Sect. B*, **26**, 765 (1970).
d Y. Fujimoto, F. Irreverre, J. M. Karle, I. L. Karle, and B. Witkop,
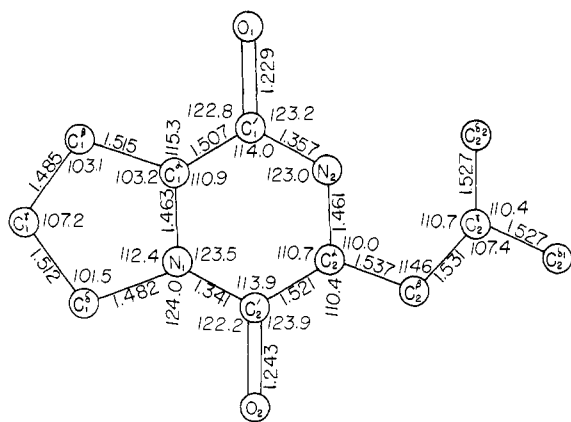*J. Amer. Chem. Soc.*, **93**, 3471 (1971).

Figure 1. Bond lengths and angles in *cyclo*-L-Pro-L-Leu. The
standard deviations range from 0.005 Å for bonds in the diketo-
piperazine ring to 0.010 Å for C–C bonds in the side chains. For the
angles, the standard deviations range from 0.3 to 0.6°.

$2\theta(\alpha_2) - 2\theta(\alpha_1)$ scan over 2θ. The process of cutting the soft crystal
may have disturbed its regularity, since a relatively large scan of
2.4° was required and may have affected the accuracy of the in-
tensities to some degree. The scan rate was 2°/min and a back-
ground count was made for 10 sec at either end. From the sys-
tematic absences, the space group was uniquely indicated to be

$P2_12_12_1$. The cell parameters are: $a = 9.451 \pm 0.002$, $b =
19.587 \pm 0.004$, and $c = 6.340 \pm 0.002$ Å; $V = 1173.6$ Å³, $Z = 4$,
$d_{calcd} = 1.190$ g/cm³ for molecular formula $C_{11}H_{18}O_2N_2$, mol wt
210.28, mp 164–166°, $\alpha D -142°$. The linear absorption coefficient
is 6.87 cm⁻¹. Lorentz and polarization corrections were made,
but no absorption corrections were applied. The intensities for a
total of 1146 independent reflections were placed on an absolute
scale by means of a K curve and normalized structure factors, $|E|$,
were derived.

Structure Determination

Phase angles for the individual $|E_{hkl}|$'s were derived
directly from the structure factor magnitudes by means
of the symbolic-addition procedure[7] for noncentrosym-
metric space groups. An origin and enantiomorph
were arbitrarily specified by assigning the values $+\pi/2$,
$+\pi/2$, 0, and $+\pi/2$ to reflections 190, 015, 810, and
0,15,2, respectively. Unknown phase values designated
by m and s were assigned to the reflections 216 and 3,13,3
to expedite the phase determination. In the course
of the phase determination, there were multiple in-
dications that $m = 0$ and $s = \pm \pi/2$. Of the two E
maps computed with 330 individual reflections, the one
with $s = -\pi/2$ revealed the structure of the mole-
cule; however, the molecule had the wrong hand for
levopeptide units. The correct hand was obtained by
reversing the signs of the assigned phases for 0,15,2
(the reflection which was used to specify the enantio-
morph) and the symbol s.

Least-squares refinement minimizing the function
$\Sigma w(|F_o| - |F_c|)^2$, with $w = 0.5$ for $F_o = 0$, $w = 1$ for
$|F_o| < 10$, and $w = 10/|F_o|$ for $|F_o| \geq 10$, resulted in a
final R factor of 6.4% for all the data.[8] The atomic
scattering factors used are those tabulated in the In-
ternational Tables for X-ray Crystallography. The
coordinates and anisotropic thermal factors for the C,
N, and O atoms and the coordinates for the H atoms
were refined. It was assumed that the thermal factors
for the H atoms were equal to those of the atom of at-
tachment. Fractional coordinates are listed in Tables
II and III, and the bond lengths and angles are shown
in Figure 1.

Discussion

The conformation of the molecule is illustrated in the
stereo diagram in Figure 2. The diketopiperazine
ring is folded. It is composed of two nearly planar
peptide units (the largest deviation of any atom from
its least-squares plane is 0.05 Å) in the cis conformation,
with a dihedral angle of 143° between the planes. The
fold occurs along the line joining the two tetrahedral
C^α atoms. The H atoms on C_1^α and C_2^α are in the axial
positions and are on the same side of the DKP ring.
Even though the side groups are dissimilar, bond lengths
and angles for the two peptide units in the DKP ring
are very similar and their torsional angles, Figure 3
and Table IV, are almost identical. The parameters
and conformation for the DKP ring are the same, within
the standard deviations, as found for V. The main
differences between the folded DKP rings in IV and V
(boat) and the DKP rings in I and II (planar) and in III

(7) See e.g. J. Karle and I. L. Karle, *Acta Crystallogr.*, **21**, 849 (1966).
(8) Observed and calculated structure factors will appear following
these pages in the microfilm edition of this volume of the journal.
Single copies may be obtained from the Reprint Department, ACS Pub-
lications, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by re-
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check or money order for $3.00 for photocopy or $2.00 for microfiche.

Table II. Fractional Coordinates and Thermal Parameters[a,b]

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
N_1	0.6236 (4)	0.1443 (2)	0.2503 (6)	4.99 (15)	3.57 (16)	3.21 (17)	0.17 (14)	−0.59 (16)	−0.27 (15)
O_1	0.3385 (4)	0.2262 (2)	−0.0074 (6)	4.57 (14)	8.04 (22)	2.77 (13)	0.72 (14)	−0.78 (14)	−0.61 (16)
C_1'	0.4467 (5)	0.2212 (2)	0.0988 (7)	3.55 (15)	5.20 (16)	2.53 (17)	0.00 (15)	0.21 (17)	0.23 (18)
C_1^α	0.5597 (5)	0.1689 (2)	0.0542 (7)	4.43 (17)	4.42 (16)	2.45 (17)	−0.38 (16)	0.10 (16)	−0.16 (16)
C_1^β	0.5085 (6)	0.1039 (3)	−0.0506 (9)	6.45 (23)	5.69 (23)	4.56 (27)	−0.51 (21)	−0.22 (25)	−1.44 (23)
C_1^γ	0.6133 (9)	0.0523 (3)	0.0205 (12)	9.39 (36)	6.00 (30)	8.57 (46)	1.73 (28)	−2.31 (40)	−3.42 (33)
C_1^δ	0.6536 (7)	0.0701 (3)	0.2448 (10)	6.38 (24)	4.33 (22)	5.77 (21)	0.69 (21)	−0.65 (19)	−0.77 (23)
N_2	0.4737 (4)	0.2610 (2)	0.2692 (6)	4.13 (14)	4.56 (15)	2.21 (15)	0.54 (14)	−0.43 (15)	−0.49 (14)
O_2	0.7223 (4)	0.1641 (2)	0.5697 (5)	5.12 (13)	4.80 (17)	3.29 (15)	0.52 (14)	−1.38 (15)	0.21 (13)
C_2'	0.6565 (5)	0.1852 (2)	0.4128 (8)	3.61 (15)	4.04 (18)	3.61 (20)	−0.03 (16)	−0.03 (19)	−0.07 (18)
C_2^α	0.6071 (5)	0.2586 (2)	0.3854 (7)	3.69 (15)	4.08 (15)	2.16 (17)	−0.12 (14)	0.09 (15)	−0.16 (15)
C_2^β	0.5898 (5)	0.2932 (2)	0.6014 (7)	4.68 (18)	4.24 (19)	2.34 (17)	0.26 (15)	−0.45 (19)	−0.59 (15)
C_2^γ	0.5774 (6)	0.3711 (2)	0.5928 (8)	5.24 (20)	4.29 (19)	3.81 (19)	0.12 (15)	−1.01 (23)	−0.70 (19)
$C_2^{\delta_1}$	0.5549 (8)	0.3962 (3)	0.8180 (9)	8.59 (30)	7.00 (30)	4.20 (26)	1.20 (28)	−0.69 (29)	−2.40 (25)
$C_2^{\delta_2}$	0.7112 (8)	0.4027 (3)	0.4992 (12)	8.33 (33)	5.62 (28)	7.17 (37)	−1.82 (28)	−0.05 (37)	−0.20 (30)

[a] The thermal parameters are expressed in the form $T = \exp[-\frac{1}{4}(B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)]$, where the B_{ij} values are in ångströms squared. [b] The standard deviations are listed in parentheses.
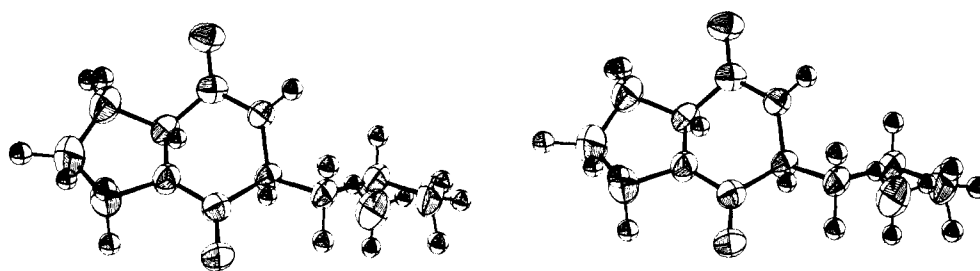

Figure 2. A stereo diagram of the configuration of cyclo-L-Pro-L-Leu. The thermal ellipsoids are drawn at the 50% probability level. The figure was drawn by computer from a program prepared by C. K. Johnson (Oak Ridge National Laboratory) and should be viewed with a three-dimensional viewer for printed stereographs.

Table III. Coordinates for Hydrogen Atoms

H atom on	x	y	z
C_1^α	0.6312	0.1910	−0.0137
C_1^β	0.4926	0.1108	−0.2134
C_1^β	0.4140	0.0890	0.0135
C_1^γ	0.5733	−0.0044	0.0261
C_1^γ	0.7146	0.0576	−0.0852
C_1^δ	0.5597	0.0457	0.3346
C_1^δ	0.7607	0.0653	0.2664
N_2	0.4063	0.2931	0.3091
C_2^α	0.6872	0.2810	0.3206
C_2^β	0.5085	0.2745	0.6574
C_2^β	0.6794	0.2774	0.6883
C_2^γ	0.4854	0.3841	0.5077
$C_2^{\delta_1}$	0.4623	0.3763	0.8907
$C_2^{\delta_1}$	0.5640	0.4526	0.8219
$C_2^{\delta_1}$	0.6509	0.3906	0.8886
$C_2^{\delta_2}$	0.7212	0.4513	0.5260
$C_2^{\delta_2}$	0.6806	0.4039	0.3487
$C_2^{\delta_2}$	0.8071	0.3879	0.5678
Std dev	0.0040	0.0020	0.0060

Table IV. Torsional Angles[a] (deg) for cyclo-L-Pro-L-Leu

	$j = 1$	$j = 2$
ω_j	+6.3	+6.2
ψ_j	+33.7	+33.8
ϕ_j	−41.5	−41.5
χ_{1j}	−31.5	−72.3
χ_{2j}	+36.0	+177.7
χ_{3j}	−25.1	
χ_{4j}	+4.5	

[a] The convention followed is that proposed by the IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471 (1970).
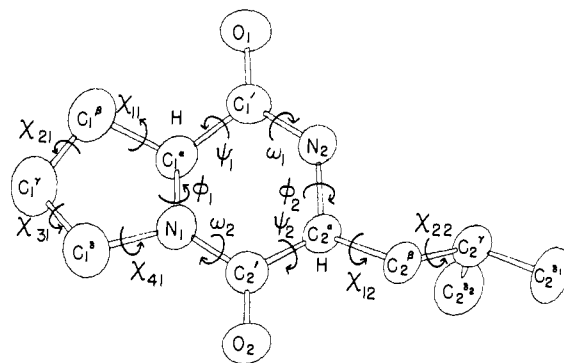

Figure 3. Labeling of atoms and rotational angles.

(twist boat) occur in the values of their $C^\alpha C' N$ and $C' N C^\alpha$ angles, which are 3–5° smaller in IV and V.[9]

The pyrrolidine ring has the envelope conformation, with atoms N_1, C_1^α, C_1^γ, and C_1^δ in a plane (to within ±0.026 Å) and C_1^β 0.52 Å out of the plane. As already mentioned in the introduction, all the *linear* polypeptides containing the prolyl group whose crystal structures have been determined have C^γ out of the plane in the pyrrolidine ring. In this molecule, the nearest H···H approaches in the pyrrolidine ring range from 2.19 to 2.48 Å, with the smallest values between the H atoms on C_1^γ and C_1^δ. The planar portion of the pyrrolidine ring is approximately coplanar with the ad-

(9) In cyclo-glycyl-L-tyrosyl, the DKP ring also has the boat conformation although considerably flatter than in cyclo-L-Pro-L-Leu. However, in cyclo-Gly-L-Tyr, the DKP ring is buckled in the opposite direction, with the benzyl constituent on C_2^α quasi-axial and the H atom equatorial: L. E. Webb and C. Lin, *J. Amer. Chem. Soc.*, **93**, 3818 (1971).

Karle / *Structure and Conformation of cyclo-L-Prolyl-L-leucyl*

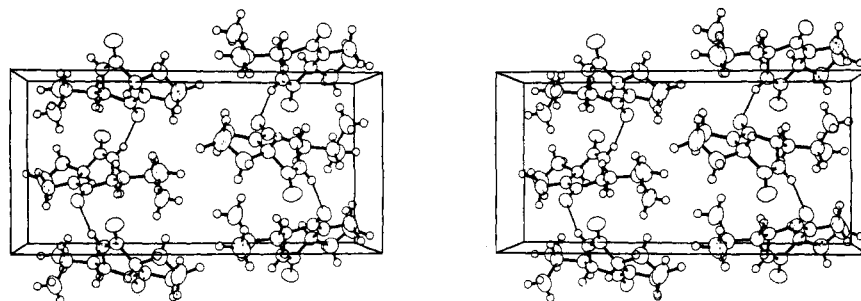Figure 4. The molecular packing. Axial directions are a, $\downarrow$; b, $\rightarrow$; and c, up from the plane of the paper. The light lines represent the NH···O hydrogen bonds.

jacent peptide unit containing C_1^α, N_1, C_2', O_2, and C_2^α. The intramolecular approaches $O_1\cdots C_1^\beta$, $O_2\cdots C_2^\beta$, and $O_2\cdots C_1^\delta$ are 2.897, 2.830, and 2.838 Å, respectively, as compared to 2.820 and 2.822 Å for O···CH$_3$ in III.

The leucyl chain is extended, with C_2^α, C_2^β, C_2^γ, and $C_2^{\delta_1}$ coplanar within 0.016 Å. All the H atoms, including those on $C_2^{\delta_1}$, are in the trans conformation with respect to each other. On the leucyl side of the molecule, the 14 nearest intramolecular H···H approaches range between 2.31 and 2.59 Å except for $H(C_2^\gamma)\cdots H(C_2^{\delta_2})$, which is ~2.14 Å. The $C_2^\alpha C_2^\beta C_2^\gamma$ angle is 114.6°, a value commonly found for the C^β angle in amino acids. The increase in this angle over a tetrahedral value is needed to separate sufficiently $H(N_2)$ from $H(C_2^\gamma)$ and $H(C_2^\alpha)$ from $H(C_2^{\delta_2})$.

The molecular packing is illustrated in Figure 4. The only possible hydrogen bond, $N_2H\cdots O_2'$ where O_2' is related to O_2 by the symmetry operation $-\frac{1}{2}+x$, $\frac{1}{2}-y$, $1-z$, connects the molecules into infinite chains parallel to the a axis. The length of the $N_2H\cdots O_2'$ bond is 2.97 Å. Aside from the hydrogen bond, the closest intermolecular approaches are at normal van der Waals values. They are between the chains in the c direction, where $O_1\cdots C_1^\alpha$, $O_1\cdots C_2^\alpha$, and $O_2\cdots C_1^\beta$ are 3.26, 3.35, and 3.36 Å, respectively, and between the chains in the b direction, where $C_1^\gamma\cdots C_1^\delta$ is 3.69 Å. Values for the thermal parameters are largest for the atoms at the periphery of the molecule, that is, for O_1, which is not involved in hydrogen bonding, and for C_1^γ, $C_2^{\delta_1}$, and $C_2^{\delta_2}$.

The Influence of Solvent on Ion Association. II. Proton Nuclear Magnetic Resonance of $[(n\text{-}C_4H_9)_4N][(C_6H_5)_3PCoBr_3]$

Yau-Yan Lim[1] and Russell S. Drago

Contribution from the W. A. Noyes Laboratory, University of Illinois, Urbana, Illinois 61801. Received January 13, 1971

Abstract: The ion pairing interaction in $[(n\text{-}C_4H_9)_4N][(C_6H_5)_3PCoBr_3]$ has been studied by proton magnetic resonance spectroscopy in a series of different solvents. The observed paramagnetic resonance shifts of the N-methylene protons of the ammonium ion were determined for a 0.058 M solution in nine different solvents with the dielectric constant ranging from 4.65 to 64.2. By concentration *vs.* chemical shift studies, the concentration association constants and the isotropic resonance shifts of the N-methylene protons in the fully ion-paired species were determined in each of the following solvents: *o*- and *m*-nitrotoluenes, nitrobenzene, and propylene carbonate. The concentration association constants were found to be constant over a concentration range of 0.02–0.5 M in electrolyte and to increase with a decrease in dielectric constant of the solvent. This finding is used to challenge the common procedure of making activity corrections to spectrophotometric data. The N-methylene proton isotropic resonance shifts of the fully ion-paired species were the same within experimental error in the former three solvents and smaller in propylene carbonate. The smaller shift in propylene carbonate ($\epsilon = 63.0$) was interpreted to indicate a longer interionic distance for the ion pair in the higher dielectric solvent.

As part of our continued interest in solvent effects[2] and the application of nmr dipolar or "pseudo-contact" shifts to the studies of ion pairing in solution,[3-5] we have examined the ion pairing interaction of

$[(C_4H_9)_4N][(C_6H_5)_3PCoBr_3]$ as a function of solvent. In contrast to the earlier study[4] on $[(C_8H_{17})_3NCH_3]_3$-$Fe(CN)_6$, the system we have chosen for the present study has the advantage that it is a simpler 1:1 electrolyte in polar solvents.

$$[(C_4H_9)_4N][(C_6H_5)_3PCoBr_3] \;\rightleftharpoons\;$$
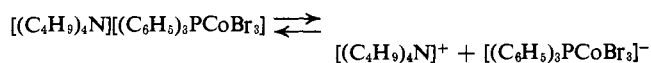$$[(C_4H_9)_4N]^+ + [(C_6H_5)_3PCoBr_3]^-$$

(1) Abstracted in part from the Ph.D. Thesis of Y. Y. Lim, University of Illinois, Urbana, Ill., 1971.

(2) See, for example, R. S. Drago and K. F. Purcell, *Progr. Inorg. Chem.*, **6**, 271 (1964).

(3) I. M. Walker and R. S. Drago, *J. Amer. Chem. Soc.*, **90**, 6951 (1968).

(4) J. C. Fanning and R. S. Drago, *ibid.*, **90**, 3987 (1968).

(5) D. G. Brown and R. S. Drago, *ibid.*, **92**, 1871 (1970).