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Reduction by a Model of NAD(P)H

XI. Stereochemistry of a Lactate Dehydrogenase-Model Reaction

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Stereochemistry of the biomimetic reduction of α-keto esters with NAD(P)H-model compounds has been investigated. The model compound with the *R*-configuration reduces the α-keto esters to the (*R*)-α-hydroxy esters, whereas (*S*)-α-hydroxy esters are afforded by the reduction with the *S*-configurational model compounds. It has been concluded that pro-*R* and -*S* hydrogens of the model compounds with *R*- and *S*-configuration, respectively, contribute predominantly to the reduction.

It has been reported that 1-benzyl-1,4-dihydronicotinamide or *N*-α-methylbenzyl-1-propyl-1,4-dihydronicotinamide (**1b**), reduces ethyl benzoylformate or butyl pyruvate with the assistance of magnesium perchlorate (*1*). The reduction is stereospecific; namely, (*R*)-**1** yields (*R*)-(−)-α-hydroxy ester, whereas (*S*)-**1** predominantly affords (*S*)-(+)-α-hydroxy ester. The reaction is the first stereochemical model of enzymic NAD(P)H-dependent reductions in the sense that a substrate discriminates between the pro-*R* and -*S* hydrogens at the C_4-position of dihydropyridine ring (*2*). Although the most straightforward method for distinguishing these two hydrogens is isotope-labeling, there is no device at present to label them in a model compound stereospecifically. Therefore, we attempted to estimate the molecular arrangement in the transition state of the reaction from the stereochemical results with various R″ groups in the model compound and various R and R′ groups in α-keto esters, **2**.

RESULTS AND DISCUSSION

All reactions were carried out at 30°C for 44 hr in the dark under an atmosphere of argon. Yields of α-hydroxyesters were greater than 97%, based on the reacted α-keto esters, although the conversion of α-keto esters changed from 60 to 100% depending on the substituents R and R′. Other products were not detected by vpc and tlc.

Table 1 indicates the variation in optical yield (percentage of enantiomer excess) of the reaction with the variation of R″. It is evident that the yield increases with the

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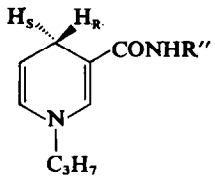
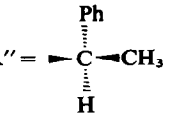
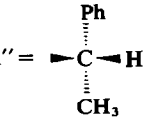
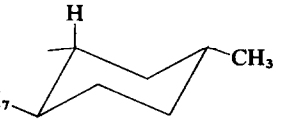


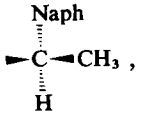
1

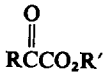
a: R″ = H

(*R*)-**b**: R″ = 

(*S*)-**b**: R″ = 

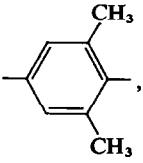
(*R*)-**c**: R″ = *i*-C_3H_7 

(*R*)-**d**: R″ =  ,



2

a: R = CH_3, R′ = $(CH_3)_2CHCH_2$
b: R = Ph, R′ = CH_3
c: R = Ph, R′ = C_2H_5
d: R = Ph, R′ = (*R*)-(−)-Menthyl

e: R =  , R′ = CH_3.

f: R =  , R′ = CH_3

g: R = $(CH_3)_3C$–, R′ = CH_3

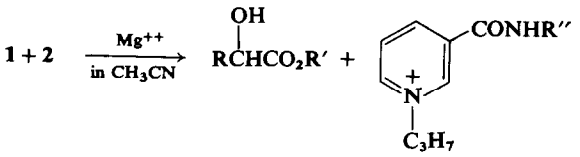


TABLE 1

VARIATION OF OPTICAL YIELD WITH THE CHANGE OF R″

		Product	
2	**1**	Configuration[a]	Enantiomer excess (%)
d	**a**	*R*	8
d	*racemic*-**b**	*R*	10
d	(*R*)-**c**	*R*	24
c	(*R*)-**b**	*R*	13
c	(*R*)-**c**	*R*	26
c	(*R*)-**d**	*R*	28

[a] Configuration at the carbon substituted by a hydroxy group.

increase of the steric bulk of R″, which clearly suggests that the C_3-side of the dihydropyridine ring in **1** is larger in bulk than the C_5-side with respect to the prochiral center at the C_4-position (*3*).

In Table 2 the results of the variation of R′ are listed. Since the menthyl group in **2d** has chiral centers in itself, it is interesting to estimate the extent of the contribution of the

TABLE 2

VARIATION OF OPTICAL YIELD WITH THE CHANGE OF R′

		Product	
2	**1b**	Configuration[a]	Enantiomer excess (%)
b	*R*	*R*	15
b	*S*	*S*	14
c	*R*	*R*	13
c	*S*	*S*	15
d	*R*	*R*	25
d	*racemic*	*R*	10
d	*S*	*R*	2

[a] Configuration at the carbon substituted by a hydroxy group.

chiral α-methylbenzyl group in **1b** to the asymmetric synthesis of an enantiomer. However, the difference in enantiomer excess between the reactions with *R*- and *racemic*-**1b** is only 15%, which is identical to those obtained from the reactions of **2b** and **2c** with (*R*)- or (*S*)-**1b**. Much higher double-asymmetric induction would be anticipated

if R′ played an important role in chiral recognition (*4*).[2] Instead, the result seems to reveal that the carboalkoxy group in **2** is situated at an open face of **1b** in the transition state of the reaction. In the reaction with **2d**, the variation of R″ ((*R*)-α-methyl benzyl to menthyl, and a hydrogen to *racemic*-α-methyl benzyl) does not affect the optical yield (cf. also Table 1).[2] This fact suggests that the critical bulk of the menthoxycarbonyl group is large enough to overcome the steric difference in R″ (*3*) in accordance with the above conclusion.

The same conclusion can be obtained from the results shown in Table 3, where the steric bulk of R is compared. It has generally been accepted, in asymmetric reduction of

TABLE 3

VARIATION OF OPTICAL YIELD WITH THE CHANGE OF R

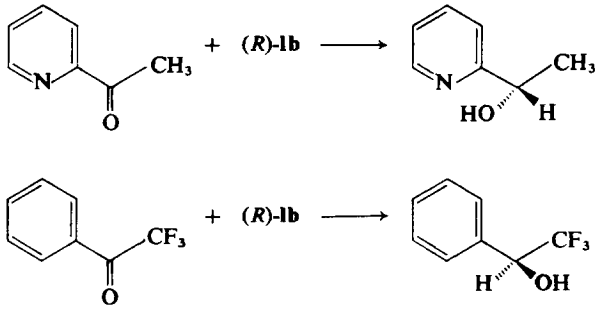
		Product	
2	**1b**	Configuration[a]	Enaniomer excess (%)
a	*R*	*R*	17
b	*R*	*R*	16
b	*S*	*S*	14
e	*R*	(*R*)[b]	—[d]
e	*S*	(*S*)[c]	—[d]
f	*R*	*R*	7
f	*S*	*S*	7
g	*R*	*R*	7
g	*S*	*S*	6

[a] Configuration at the carbon substituted by a hydroxy group.
[b] $[\alpha]_D^{27}$ −16.9.
[c] $[\alpha]_D^{27}$ +19.8.
[d] Optical rotation of the pure compound is not known.

ketones, that the steric bulk of groups decreases in this order: Ph > *t*-Bu > *cyclo*-C_6H_{11} > Et > Me (*3*). The present results may be interpreted with this decreasing order, Ph > *t*-Bu ≈ *cyclo*-C_6H_{11}, provided one assumes larger bulk for R than for CO_2R'. However, this order does not predict the *R*-configuration for the product of the reaction of **2a** with (*R*)-**1b**: one has to propose larger steric bulk for the methyl group than for the carbo-*iso*-butoxy group in order to predict the predominant formation of (*R*)-*iso*-butyl lactate. On the other hand, when a decreasing order of bulkiness, CO_2R' > *t*-Bu ≈ *cyclo*-C_6H_{11} > Ph > CH_3, is assumed (*5*), the result may be logically interpreted: the greater the difference in steric bulk between R and CO_2R', the greater will be the optical

[2] This discussion does not deny that the interaction causes the double-asymmetric induction. The reaction of **2d** with (*S*)-**1b** is expected to afford 10% (*R*) minus 15% (*S*) equaling a 5% excess of the (*S*)-isomer, whereas the experimental result shows that the product is composed of an excess of the (*R*)-isomer; apparently an (*R*)-configurational R″ and achiral or *racemic*-R″ result in different optical yields.

yield (*3*). The results listed in Table 2 also agree with the assumed steric order (CO_2R' > R). It should be noted that the chiral center in the model compound is separated from the reaction center by five atoms, and the effective bulk of a substituent in the present reaction may be different from those in other reactions, where a chiral center is, in general, adjacent to the reaction center. Thus, the tail-part of a substituent may contribute to the chiral recognition in the present reaction more than, or as well as, the head-part. Although we have discussed the stereochemistry of the reaction from the viewpoint of steric factors alone, it is apparent that electronic effects have to be taken into account as well. It is quite possible that some electronic interaction (attraction or repulsion) between functional groups in the model compound and the substrate play the most important role in effecting molecular arrangement, as concluded above. The formations of (*S*)-(+)-1-(2′-pyridyl)ethanol from 2-acetylpyridine (*6*) and (*S*)-(+)-pheny trifluoromethyl carbinol from α,α,α-trifluoroacetophenone (*7*) by the reductions with (*R*)-**1b** reveal the importance of electronic effects. A similar contradiction has also been observed in enzymic systems (*8*,*9*).



The role of the magnesium ion has not yet been clarified. However, it seems likely that the magnesium ion enhances the steric and/or electronic effect operating in the present reaction.[3]

Consequently, the molecular arrangement in the transition state of the reduction of (at least) α-keto esters may be represented by Fig. 1 (*7*), from which it is inevitably

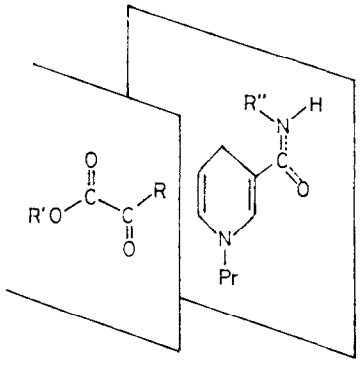


FIG. 1. Schematic representation of the transition state. The position of the magnesium ion is ambiguous.

[3] The molar ratio of magnesium ion to substrate defines the amount of stereospecificity (*6*). The reaction is non-stereospecific with lithium perchlorate.

deduced that the pro-*R* and -*S* hydrogens contribute predominantly to the reduction with (*R*)- and (*S*)-**1**, respectively.

Corey–Pauling–Koltun models for (*R*)- and (*S*)-**1** indicate that pro-*R* and -*S* hydrogens in (*R*)- and (*S*)-model compounds, respectively, are less hindered than the others.

EXPERIMENTAL

Acetonitrile was distilled once over phosphorus pentoxide and kept over 4A molecular sieves. Anhydrous magnesium perchlorate was kept in a vacuum desiccator over phosphorus pentoxide. Optically active amines were purchased from Norse Laboratories. Ethyl benzoylformate, bp 118°C/5 mm Hg (lit. (*10*) bp 118°C/5 mm Hg), was obtained from a commercial source (Tokyo-kasei Co.) and was purified by distillation.

1-Propyl-1,4-dihydronicotinamide (**1a**)

This compound, mp 86°C (decomposition) (lit. (*12*) mp 86°C), was prepared according to Mauzerall and Westheimer (*11*).

N-α-Methylbenzyl-1-propyl-1,4-dihydromicotinamide (**1b**)

Into a pyridine (50 ml) solution of nicotinyl chloride hydrochloride (8.5 g) (*13*), 7 g of α-methylbenzylamine in 25 ml of pyridine at room temperature was added dropwise. The mixture was kept at 85°C for 1 hr and cooled to a room temperature, then was diluted with a saturated aqueous sodium bicarbonate. Organic materials were extracted with methylene chloride and the organic layer was washed with water, dried over Drierite, and concentrated *in vacuo*. The residual brown oil was dissolved in ether containing a small amount of ethanol and repeatedly treated with activated charcoal. Recrystallizations from the same solvent gave 5 g (45% yield) of *N*-α-methylbenzoylnicotinamide, mp 82°C (lit. (*14*) mp 82°C).

The crystals thus obtained (3 g) and propyl iodide (6 g) in 30 ml of ethanol were heated to reflux for 5 hr on an oil bath, and the low-boiling materials were evaporated *in vacuo*. The residual viscous oil was washed twice with ether and dried in a desiccator.

Into 100 ml of an aqueous solution containing 10 g of sodium carbonate and 10 g of sodium dithionite, an ethanol solution (30 ml) of the viscous oil at room temperature was added. The mixture was kept in a refrigerator for a while and was poured into a large amount of cold water. The crystals which appeared were filtered and recrystallized from 50% aqueous ethanol, affording 1.3 g (37% yield) of **1b**: mp 110°C (decomposition); nmr ($\delta^{TMS}_{CDCl_3}$) 0.88 (*t*, 3*H*), 1.4–1.7 (*d* + *q*, 2*H*), 2.98 (*t*, 2*H*), 3.09 (*q*, 2*H*), 4.60 (*d* of *t*, 1*H*), 6.0–6.4 (*bd* + quint, 2*H*), 5.66 (*d* of *t*, 1*H*), 6.78 (*d*, 1*H*), and 7.27 (*m*, 5*H*).

Anal. Calcd for $C_{17}H_{22}N_2O$: C, 75.52; H, 8.20; N, 10.36. Found: C, 75.61; H, 8.09; N, 10.65.

(*R*)-(+)-α-Methylbenzylamine ($[\alpha]_D^{20}$ +35.9, neat) gave (*R*)-(−)-**1b** ($[\alpha]_D^{24}$ −172.9 (*c*, 2.00, MeCN)), whereas (*S*)-(−)-α-methylbenzylamine ($[\alpha]_D^{20}$ −36.0, neat) yielded (*S*)-(+)-**1b** ($[\alpha]_D^{24}$ +173.3 (*c*, 2.00, MeCN)).

N-Menthyl-1-propyl-1,4-dihydronicotinamide (**1c**)

By starting with 6.8 g of (*R*)-(−)-menthylamine ($[\alpha]_D^{20}$ −35.2, neat) and 8 g of nicotinyl chloride hydrochloride, 4.6 g (40% yield) of *N*-menthylnicotinamide, mp 132.5–133.5°C, was obtained after treatment similar to that mentioned above.

Anal. Calcd for $C_{16}H_{24}N_2O$: C, 73.81; H, 9.29; N, 10.76. Found: C, 74.07; H, 9.20; N, 10.66.

The successive reactions of *N*-menthylnicotinamide with propyl iodide and sodium dithionite gave **1c** (35% yield): mp 96–98°C (decomposition); $[\alpha]_D^{31}$ −102.1 (*c*, 1.48, MeCN); nmr ($\delta_{CDCl_3}^{TMS}$) 0.5–2.1 (*m*, 23*H*), 2.99 (*t*, 2*H*) 3.08 (*q*, 2*H*), 3.84 (*m*, 1*H*), 4.59 (*d* of *t*, 1*H*), 4.76 (*m*, 1*H*), 5.67 (*d* of *q*, 1*H*), and 6.97 (*d*, 1*H*).

Anal. Calcd for $C_{19}H_{32}N_2O$: C, 74.95; H, 10.59; N, 9.20. Found: C, 74.83; H, 10.58; N, 8.96.

N-α-(1′-Naphthylethyl)-1-propyl-1,4-dihydronicotinamide (**1d**)

The reaction with 22.9 g of (*R*)-(+)-α-(1′-naphthylethyl)amine ($[\alpha]_D^{20}$ +78, neat) and 24.3 g of nicotinyl chloride hydrochloride afforded 16.7 g (49% yield) of *N*-α-naphthylethylnicotinamide, mp 158.5–159.5°C.

Anal. Calcd for $C_{18}H_{16}N_2O$: C, 78.24; H, 5.84; N, 10.14. Found: C, 78.45; H, 5.82; N, 10.21.

N-α-(1′-naphthylethyl)nicotinamide was reacted successively with propyl iodide and sodium dithionite to give **1d** (25% yield): mp 140–142°C (decomposition); $[\alpha]_D^{29}$ −280.5 (*c*, 0.80, MeCN); nmr ($\delta_{CDCl_3}^{TMS}$) 0.84 (*t*, 3*H*), 1.48 (sext, 2*H*), 1.61 (*d*, 3*H*), 2.93 (*t*, 1*H*), 2.94 (*q*, 2*H*), 4.50 (*d* of *t*, 1*H*), 5.37 (*bd*, 1*H*), 5.59 (*d* of *q*, 1*H*), 5.96 (quint, 1*H*), 6.99 (*d*, 1*H*), 7.3–7.6 (*m*, 4*H*), 7.64–7.9 (*m*, 2*H*), and 8.0–8.1 (*m*, 1*H*).

Anal. Calcd for $C_{21}H_{24}N_2O$: C, 78.71; H, 7.55; N, 8.74. Found: C, 78.46; H, 7.55; N, 8.58.

Iso-Butyl pyruvate (**2a**)

Pyruvic acid was reacted with *iso*-butanol (*15*), bp 80°C/20 mm Hg (lit. (*15*) bp 80°C/20 mm Hg).

Methyl benzoylformate (**2b**)

Benzoylformic acid was esterified with diazomethane, bp 112°C/6 mm Hg (lit. (*16*) bp 110–111°C/6 mm Hg).

Menthyl benzoylformate (**2d**)

Oxidation of menthyl mandelate with lead tetraacetate (*16*) gave **2d**: mp 73–74°C (lit. (*17*) mp 72–73°C); $[\alpha]_D^{27}$ −44.2 (*c*, 1.00, EtOH) ($[\alpha]_D^{26}$ −45.7 (*c*, 4.85, EtOH)).

Methyl 2,4,6-trimethylbenzoylformate (**2e**)

2,4,6-Trimethylacetophenone (*18*) was oxidized to 2,4,6-trimethylbenzoylformate (*18*, *19*), which was treated with diazomethane to give **2e**, bp 82–83°C/5 mm Hg (lit. (*19*) bp 170°C/100 mm Hg).

Methyl hexahydrobenzoylformate (**2f**)

A 150-ml ethereal solution of a Grignard reagent, prepared from 3.6 g of magnesium and 25 g of cyclohexyl bromide, was added dropwise at 10°C to 500 ml of an ethereal solution of dimethyl oxalate (60 g). The reaction mixture was kept at room temperature for 1 hr, then refluxed for 2 hr. After the usual work-up, the excess dimethyl oxalate was filtered off. The filtrate was distilled to give **2g**: bp 59–60°C/2.2 mm Hg; nmr ($\delta_{CDCl_3}^{TMS}$) 1.0–2.2 (*m*, 10*H*), 2.7–3.3 (*m*, 1*H*), and 3.85 (*s*, 3*H*).

Anal. Calcd for $C_9H_{14}O_3$: C, 63.51; H, 8.29. Found: C, 63.51; H, 8.57.

Methyl trimethylpyruvate (**2g**)

t-Butyl methyl ketone was oxidized to trimethylpyruvic acid (*20*), which was converted into the methyl ester, bp 59–60°C/18 mm Hg (*lit.* (*21*) bp 69–70°C/20 mm Hg).

General Procedure

An aliquot composed of 1 mmol of an α-keto ester, 1 mmol of a model compound, and 2 mmol of magnesium perchlorate in 10 ml of acetonitrile was stirred for 44 h rat 30°C under an atmosphere of argon in the dark. The esters **2a–2d** converted 100% into the corresponding α-hydroxy esters. The conversions for **2e–2g** were, however, 60–70%. The yield of the product was more than 97% in each run.

The reaction mixture was stirred for 5 min at room temperature after the addition of 1 ml of water, and the product was extracted with methylene chloride. The solvent was evaporated *in vacuo* at temperatures below 30°C. The residue was chromatographed on a column of silica gel with benzene or benzene–ethyl acetate (85:15, v/v) as an eluent. The optical activity of the α-hydroxy ester was measured on a Perkin–Elmer 241 Polarimeter and (in part) on a Varian HA-100 or T-60 nmr spectrometer. The purities of α-keto

TABLE 4

OPTICAL ACTIVITIES OF α-HYDROXY ESTERS, $RCH(OH)CO_2R'$

R	R′	Configuration[a]	$[\alpha]_D$	Temperature (°C)	Solvent	Concentration (*M*)	Reference
CH_3	*iso*-Bu	*S*	−15.4	18	Neat	—	(*22*)
Ph	CH_3	*R*	−142	—	MeOH	3	(*23*)
Ph	C_2H_5	*R*	−104	24	EtOH	3.29	(*24*)
Ph	(−)-Menthyl	*R*	−138.6	17	EtOH	4.37	(*25*)
		racemic	−74.2	18	EtOH	3.60	(*25*)
		S	−7.6	10	EtOH	—	(*25*)
cyclo-C_6H_{11}	CH_3	*R*	−24.3	20	Neat	—	(*26*)
t-Bu	CH_3	*R*	−31.2	22	Neat	—	(*27*)

[a] Configuration at the carbon substituted by a hydroxy group.

and α-hydroxy esters were confirmed on a Yanagimoto G-1800 gas chromatograph (5% PEG, 1 m) and by elemental analyses. Optical activities of pure α-hydroxy esters were obtained from the literature as listed in Table 4. The optical yields were calculated from the values obtained under the same conditions listed in Table 4. At least three runs were repeated for each reaction, and the absolute error in $[\alpha]_D$ was estimated to be ±2°.

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REFERENCES

1. Y. OHNISHI, M. KAGAMI, AND A. OHNO, *J. Amer. Chem. Soc.* **97**, 4766 (1975).
2. For example, see R. BENTLEY, "Molecular Asymmetry in Biology," Vol. 2, pp. 1–89. Academic Press, New York, 1970.
3. J. D. MORRISON AND H. S. MOSHER, "Asymmetric Organic Reactions," Chapter 5. Prentice-Hall, Englewood Cliffs, N.J., 1971.
4. A. HOREAU, H. B. KAGAN, AND J. P. VIGNERON, *Bull. Soc. Chim. France*, 3795 (1968).
5 Relatively small bulk of the phenyl group has been proposed: see Y. KODAMA, Y. IITAKA, K. NISHIHATA, AND M. NISHIO, *J. Chem. Soc. Parkin II*, in press.
6. Y. OHNISHI, T. NUMAKUNAI, T. KIMURA, AND A. OHNO, *Tetrahedron Lett.*, in press.
7. Y. OHNISHI, T. NUMAKUNAI, AND A. OHNO, *Tetrahedron Lett.* 3813 (1975).
8. J. EVERSE, AND N. O. KAPLAN, *Advan. Enzymol.* **37**, 61 (1973).
9. J. M. GEORGE, J. C. ORR, A. G. C. RENWICK, P. CARTER, AND L. L. ENGEL, *Bioorg. Chem.* **2**, 140 (1973).
10. B. B. CORSON, R. A. DODGE, S. A. HARRIS, AND R. K. HAZEN, *Org. Synth.* Coll. Vol. **1**, 241 (1941).
11. D. MAUZERALL AND F. H. WESTHEIMER, *J. Amer. Chem. Soc.* **77**, 2261 (1955).
12. G. STEIN AND G. STIASSNY, *Nature (London)* **176**, 734 (1955).
13. F. J. VILLANI AND M. S. KING, *Org. Synth.* Coll. Vol. **4**, 88 (1963).
14. A. N. KOST, L. A. GOLOVLEVA, P. B. TERENTÉV, A. P. TERENTÉV, AND U. M. DEINYANOVICH, *Zh. Organ. Khim.* **2**, 1700 (1966); *Chem. Abstr.* **66**, 64922f (1970).
15. I. I. LAPKIN AND O. M. LAPKINA, *Zh. Obshch. Khim.* **18**, 485 (1948); *Chem. Abstr.* **42**, 7274a (1948).
16. E. BAER AND M. KATES, *J. Amer. Chem. Soc.* **67**, 1482 (1945).
17. M. J. KUBITSCHECK AND W. A. BONNER, *J. Org. Chem.* **26**, 2194 (1961).
18. M. DITTRICH AND V. MEYER, *Liebigs Ann. Chem.* **264**, 129 (1891).
19. E. FEITH, *Ber. Deut. Chem. Ges.* **24**, 3542 (1891).
20. C. GLÜCKSMANN, *Monatsh. Chem.* **10**, 770 (1889).
21. A. RICHARD, *Ann. Chim. (Paris)* [8] **21**, 360 (1910).
22. E. WASSMER AND P. A. GUYE, *J. Chim. Phys.* 257 (1903); *Chem. Zentr.* **74**, 1418 (1903).
23. G. W. CLOUGH, *J. Chem. Soc.* 2808 (1925).
24. R. ROGER, *J. Chem. Soc.* 2168 (1932).
25. A. MCKENZIE, *J. Chem. Soc.* **85** 1249 (1904).
26. K. FREDENBERG AND L. MARKERT, *Ber. Deut. Chem. Ges.* **58**, 1753 (1925).
27. J.-P. GUETTÉ AND N. SPASSKY, *Bull. Soc. Chim. France* 4217 (1972).