biological and physiological point of view.[9] Present report is another proposal for uphill transport from the physicochemical stand point.

9) T. Rosenberg and W. Wilbrandt, *J. Theoret. Biol.*, **5**, 288 (1963).

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*UDC 615.356.011.4 : 577.164.13 : 543.422.25*

# Nuclear Magnetic Resonance Studies on Schiff Base and Related Compounds derived from Pyridoxal

Yoshikazu Matsushima[1a] and Tohru Hino[1b]

*National Institute of Radiological Sciences*[1]

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Biologically important reactions of amino acids such as transamination, racemization and so forth are catalyzed by enzymes containing pyridoxal. These reactions were found to be carried out nonenzymatically with metal ions as catalyst by Metzler, Ikawa and Snell[2] and were confirmed by many authors.[3] The first step of both enzymatic and nonenzymatic process is supposed to be reaction of amino group of amino acid and aldehyde of pyridoxal to form Schiff base.

In the former work,[4] reactions of pyridoxal with various amino acids and amines in methanol were studied by means of electronic absorption spectra. Methanol was chosen as a solvent, because Schiff base and other products were fairly stable in this solvent. Most amino acids and amines formed Schiff base as ultimate products. However, some amino acids or amines yielded products other than Schiff base, such as carbinolamine and substances having a tetrahydropyridoimidazole or a thiazolidine ring. Though biological significance of these compounds is open to future studies, some metal ion catalyzed reactions involving these compounds have been reported.[5]

In the present study, four compounds were prepared by conventional methods as examples of the products from pyridoxal and amino acids or amines; *i.e.* Schiff base of glycine (potassium pyridoxylideneglycinate), carbinolamine from sarcosine (potassium N-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridylhydroxymethyl) sarcosinate), cyclic product from histidine (potassium 4-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridyl)-4,5,6,7-tetrahydropyrido[3,4-*c*] imidazole-6-carboxylate) and thiazolidine derivative from cysteamine (2-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridyl)thiazolidine). These four compounds as well as their component substances were studied by nuclear magnetic resonance (NMR) spectroscopy, to obtain further supports for the structures established by electronic absorption spectroscopy. Most NMR studies were carried out in tetradeuteromethanol ($CD_3OD$) solutions, but trifluoroacetic acid (TFA) and hexadeuterodimethylsulfoxide (DMSO-$d_6$) were also used as solvents.

1) Location: *Anagawa, Chiba,* Present address: *a) Faculty of Pharmaceutical Sciences, Kyushu Univer-sity, Katakasu, Fukuoka; b) Faculty of Pharmaceutical Sciences, University of Chiba, Yayoi, Chiba.*
2) D.E. Metzler, M. Ikawa and E.E. Snell, *J. Am. Chem. Soc.*, **76**, 648 (1954).
3) E.E. Snell, P.M. Fasella, A. Braunstein and A. Rossi Fanelli eds., "Chemical and Biological Aspects of Pyridoxal Catalysis," Pergamon Press, Oxford, England, 1963.
4) Y. Matsushima, *Chem. Pharm. Bull.* (Tokyo), **16**, 2050 (1968).
5) Y. Matsushima, *Chem. Pharm. Bull.* (Tokyo), **16**, 2151 (1968).

## Results and Discussion

### Pyridoxal

The NMR spectrum of a $CD_3OD$ solution of pyridoxal is shown in Fig. 1. Three proton singlet at 2.47 ppm is obviously due to $C_2$ methyl proton. One proton peaks at 6.49 ppm and 7.87 ppm can be assigned to hemiacetal proton and $C_6$ proton, respectively. A quartet partially overlapped with the signal of water in the solvent is observed at around 4.97 ppm and could be assigned as $C_5$ methylene. The quartet is clearly observed in a DMSO-$d_6$ solution. This quartet and the signal at 6.49 ppm support the intramolecular hemiacetal structure of pyridoxal. Chemical shifts of protons of pyridoxal and its hydrochloride

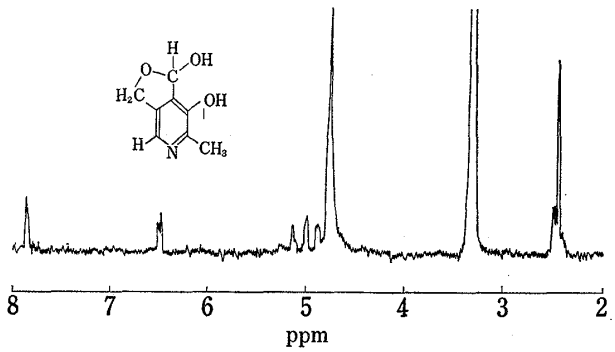



Fig. 1. NMR Spectrum of Pyridoxal in $CD_3OD$

Table I. NMR Spectra of Pyridoxal

| Chemical form | Solvent | Chemical shift[a] | | | |
|---|---|---|---|---|---|
| | | $C_2$–C**H**$_3$ | $C_5$–C**H**$_2$ | $C_4$–C**H** | $C_6$–**H** |
| Free base | $CD_3OD$ | 2.47 | 4.97 (q) | 6.49 (d) | 7.87 |
| HCl salt | $CD_3OD$ | 2.71 | 5.21 q | (6.65) | 8.20 |
| Free base | DMSO-$d_6$ | 2.41 | 4.92 q | 6.56 d | 7.91 |
| HCl salt | DMSO-$d_6$ | 2.62 | 5.07 q | 6.61 | 8.26 |
| HCl salt | TFA | 2.82 | 5.50 q | 7.58 | 8.26 |
| Cation[b] | $D_2O$ | 2.65 | 5.08 br. s | 6.71 (d) | 8.20 |

*a*) Chemical shifts are expressed by $\delta$ values (ppm) from tetramethylsilane as an internal standard. Abbreviations are as follows; br, broad; s, singlet; d, doublet; t, triplet; m, multiplet. Letters in parentheses indicate that comments are given in the text.
*b*) reference 6

in some solvents are compiled in Table I. Conversion of pyridoxal to its hydrochloride is seen to cause down field shift, which was most eminent at $C_6$ proton. This could be reasonably understood as shielding of proton is most influenced at $C_6$ by protonation of pyridine nitrogen.

The signal at 6.49 ppm in the $CD_3OD$ solution of pyridoxal is splitted further. Corresponding signal in DMSO-$d_6$ solution was observed as a doublet. Spectrum in $D_2O$ solution described by Korytnyk and Singh[6] also showed a split by 1 cps on the signal of hemiacetal proton. This might be caused by coupling with $C_5$ methylene proton, as the split of the same magnitude were observed at the quartet at 4.97 ppm.

In a $CD_3OD$ solution of pyridoxal hydrochloride, the intensity of signal of hemiacetal proton decreased and weak signals at 6.12 ppm and 6.34 ppm were observed. The upfield signals might be due to the presence of methylated hemiacetal of pyridoxal in the solution, as the hemiacetal is known to be methylated in an acidic condition.

### Schiff Base

The NMR spectrum of a $CD_3OD$ solution of potassium pyridoxylideneglycinate, shown in Fig. 2, is characterized by four singlet peaks with 3:2:1:1 intensity ratio from the upfield. The peak at 2.43 ppm is due to methyl proton. The chemical shift is the same as pyridoxal

6) W. Korytnyk and R.P. Singh, *J. Am. Chem. Soc.*, **85**, 2813 (1963).

free base, which suggests that pyridine nitrogen is not protonated in neutral methanol. This agrees with the results from electronic absorption spectra that the Schiff base existed exclusively as *keto*–enamine and *enol*–imine species in neutral methanol.[4] For $C_6$ proton 7.74 ppm peak was most suitable from comparison with spectra of pyridoxal. Therefore, 8.84 ppm peak should be assigned to azomethine proton. This assignment conforms the results of Dudek,[7] who studied NMR spectra of Schiff bases of 2-hydroxynaphthaldehyde and salicylaldehyde with amines and assigned peaks at 8.3—8.8 ppm to "aldehydic proton."

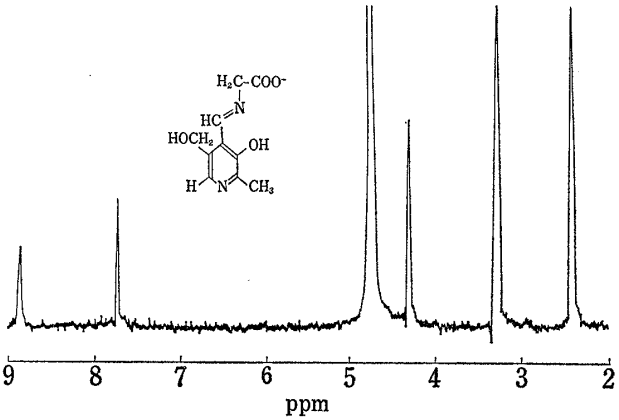



Fig. 2. NMR Spectrum of Potassium Pyridoxylideneglycinate in $CD_3OD$

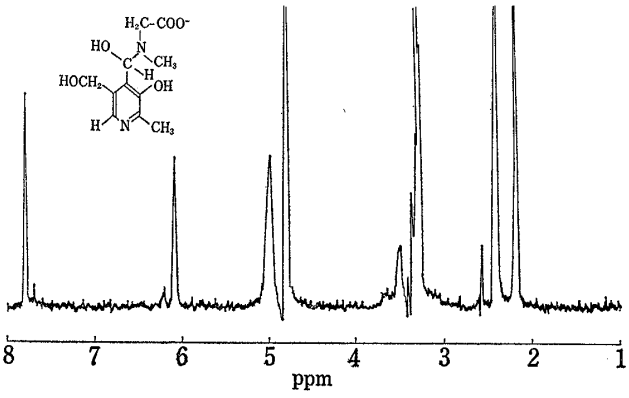



Fig. 3. NMR Spectrum of Carbinolamine in $CD_3OD$

A signal at 4.34 ppm could be assigned to methylene protons of either glycine moiety or pyridoxal moiety. In a spectrum of $CD_3OD$ solution of potassium glycinate, the signal of methylene was found at 3.15 ppm. It is reasonable to assume that Schiff base formation caused down field shift for glycine methylene to 4.34 ppm. On the other hand, protons of $C_5$ methylene were expected to show lower shift than those of pyridoxal. It is probable that the signal of the protons was hidden by the strong absorption of water proton. These assignments showed the good agreements with the results of Karpeisky,[8] who studied pyridoxal oxime and Schiff base of alanine and other amino acids in alkaline methanol.

The NMR spectrum of a DMSO–$d_6$ solution had two singlet peaks at 4.13 ppm and 4.62 ppm. The former should be assigned to protons of glycine methylene and the latter to those of $C_5$ methylene in pyridoxal moiety, which are equivalent in Schiff base due to the free rotation.

**Carbinolamine from Pyridoxal and Sarcosine**

The NMR spectrum of a $CD_3OD$ solution of potassium sarcosinate revealed that chemical shifts of protons of methyl and methylene to be 2.37 ppm and 3.09 ppm, respectively.

Carbinolamine formed from pyridoxal and potassium sarcosinate gave spectrum shown in Fig. 3 in a $CD_3OD$ solution. Three proton peaks at 2.24 ppm and 2.46 ppm are obviously due to methyl groups in sarcosine and pyridoxal moieties, respectively. Signals of methylene protons of sarcosine moiety seemed to be overlapped by signal of methyl protons of $CH_3OH$ (3.3 ppm) contained in the solvent.

Two proton peak at 5.00 ppm was assigned to $C_5$ methylene protons of pyridoxal. To be a singlet peak suggests the absence of intramolecular cyclization. Peak at 6.09 ppm was due to proton on the carbinol carbon. Upfield shift is seen from proton of hemiacetal of pyridoxal (6.49 ppm) as vicinal atom is changed from oxygen to nitrogen. That no signal

7) G.O. Dudek, *J. Am. Chem. Soc.*, **85**, 649 (1963).
8) K.F. Turchin, V.F. Bystrev, M.Ya. Karpeisky, A.S. Olkhovoy, V.L. Florentief, and Yu.N. Sheinker, "Pyridoxal Catalysis: Enzymes and Model Systems," eds. by E.E. Snell, A.E. Braunstein, E.S. Severin and Yu.M. Torchinsky, Interscience Publishers Inc., New York, N.Y., 1968, pp. 67—79.

was observed around 6.5 ppm in Fig. 3 showed dissociation of carbinolamine to pyridoxal to be negligible. Peak at 7.80 ppm is assigned to $C_6$ proton.

**Pyridoxamine**

The NMR spectrum of pyridoxamine dihydrochloride in TFA is shown in Fig. 4. Sharp singlet peaks at 2.88 ppm, 5.19 ppm and 8.44 ppm are assigned to protons of $C_2$ methyl, $C_5$ methylene and $C_6$, respectively. Peak at 4.85 ppm which has no corresponding signal in pyridoxal should be due to protons of methylene at $C_4$. Splitting might be caused by coupling with protons of vicinal $-NH_3^+$, signal of which was found at 7.68 ppm as a broad singlet.

The spectrum of a $CD_3OD$ solution of pyridoxamine dihydrochloride was very much alike to that of TFA solution, having singlet peaks at 2.80 ppm, 4.45 ppm and 8.30 ppm. But $C_5$ methylene signal was hidden by water signals and protonated amino group was not observed in this solvent.

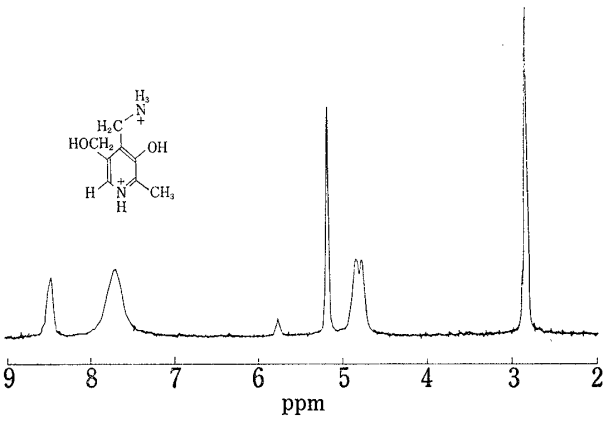



Fig. 4. NMR Spectrum of Pyridoxamine in Trifluoroacetic Acid

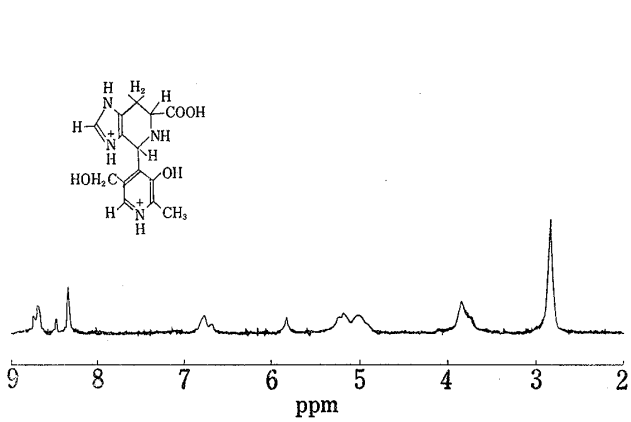



Fig. 5. NMR Spectrum of Cyclic Product in Trifluoroacetic Acid

**Cyclic Product from Pyridoxal and Histidine**

The NMR spectrum of a TFA solution of the cyclic product, shown in Fig. 5, can be interpreted from NMR data of its components, *i.e.* pyridoxal, pyridoxamine and histidine, which are tabulated in Tables I and II. The NMR spectrum of a TFA solution of histidine was reported by Bovey and Tiers[9] and we obtained the almost the same results.

Protons of the cyclic product had almost the same chemical shift as corresponding protons in pyridoxamine and histidine, as shown in Table II. A peak at 7.61 ppm observed in histidine lacked in the spectrum of the cyclic product. This clearly indicates that cyclization takes place at 5–position of imidazole ring.

Table II. NMR Spectra in Trifluoroacetic Acid Solutions

| | Chemical shifts[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Compounds | Py.[b] 2-$CH_3$ | A.A.[b] $\beta$-$CH_2$ | Py. 4-$CH_2$ | A.A. $\alpha$-CH | Py. 5-$CH_2$ | Py. 4-CH | Im.[b] 5-H | Py. 6-H | Im. 2-H |
| Pyridoxamine | 2.88 | — | 4.85 d | — | 5.19 | — | — | 8.44 | — |
| Histidine | — | 3.80 d | — | 4.85 br. s | — | — | 7.61 | — | 8.70 |
| Cyclic product | 2.82 | 3.82 br | — | 4.99 br | 5.16 br | 6.77 | — | 8.34 | 8.76 |

*a*) See footnote *a* in Table I.
*b*) Py., A.A. and Im. indicate pyridoxal, amino acid and imidazole moieties, respectively.

9) F.A. Bovey and G.V.D. Tiers, *J. Am. Chem. Soc.*, **81**, 2870 (1959).

## Thiazolidine Derivative from Pyridoxal and Cysteamine

The NMR data of $CD_3OD$ and TFA solutions of the thiazolidine derivative as well as of cysteamine are compiled in Table III. In spectra of cysteamine, two triplets for two methylene groups were observed. Upfield triplet is due to methylene protons vicinal to sulfur atom, while downfield one to those vicinal to nitrogen atom. In spectra of the thiazolidine derivative, those two absorption shifted downfield and showed complexed signals of a $A_2B_2$ type. Peaks due to pyridoxal moiety of the thiazolidine derivative could be assigned by comparison with the data on pyridoxal and pyridoxamine as shown in Table III. Every peak is seen to shift downfield in a TFA solution than in a $CD_3OD$ solution.

Table III. NMR Spectra of Cysteamine and Thiazolidine Derivative

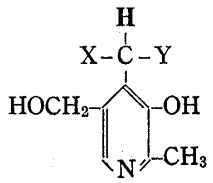
| Compounds | Solvents | Chemical shifts[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | S–$CH_2$ | N–$CH_2$ | 2–$CH_3$ | 5–$CH_2$ | 4–CH | 6–H |
| Cysteamine hydrochloride | $CD_3OD$ | 2.81 t | 3.14 t | | | | |
| | TFA | 2.98 q | 3.39 q | | | | |
| Thiazolidine derivative | $CD_3OD$ | 3.15 m | 3.57 m | 2.37 | 4.64 q | 5.93 | 7.78 |
| | TFA | 3.72 m | 4.3 m | 2.90 | 5.20 q | 6.62 | 8.42 |

a) See footnote *a* in Table I.

## Conclusion

Comparison of chemical shifts of the $C_4$ methine proton of pyridoxal derivatives is tabulated in Table IV. The Table clearly shows that the deshielding effect of hetero atom increases in the well known order S<N<O. The chemical shift of $C_4$ methine proton in the Schiff base is the lowest in the series.

Tabel IV. Chemical Shifts of $C_4$-Methine Proton of Pyridoxal Derivatives[a]




| Compounds | X | Y | Solvents | | |
|---|---|---|---|---|---|
| | | | $CD_3OD$ | TFA | DMSO–$d_6$ |
| Thiazolidine derivative | N | S | 5.93 | 6.61 | — |
| Cyclic product | N | C | — | 6.77 | 5.52 |
| Carbinolaimne | N | O | 6.09 | — | 5.93 |
| Pyridoxal hemiacetal | O | O | 6.49 | 7.58 | 6.56 |
| Pyridoxylideneglycine | N= | — | 8.84 | 8.27 | 8.70 |

a) See footnote *a* in Table I.

As shown above NMR spectra of the prepared compounds as well as their components were reasonably interpreted. Thus the structures proposed by electronic absorption spectra in the former work[4] have been given further support by NMR spectroscopy.

## Experimental

**NMR Measurements**——The spectra were obtained with a Varian HR–100 spectrophotometer, operating at 100 Mcps at room temperature. Chemical shifts are expressed by $\delta$ values (ppm) from tetramethylsilane

as an internal standard. Solutions were prepared immediately before the measurements by dissolving weighed material in solvents at low humid condition.

**Preparations**

**Schiff Base of Glycine (Potassium Pyridoxylideneglycinate)**——One mmole of pyridoxal was dissolved in 30 ml MeOH. To the pale yellow MeOH solution, MeOH solution of 1 mmole K glycinate was added slowly. The deep yellow mixture was stirred for 5 hr at room temperature and was evaporated to dryness under reduced pressure. The resulting yellow powder was recrystallized three times from MeOH. Very hygroscopic yellow crystals were obtained. *Anal.* Calcd. for $C_{10}H_{11}O_4N_2K$: N, 10.68. Found: N, 10.51.

**Carbinolamine (Potassium N-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridylhydroxymethyl)-sarcosinate)**—Aqueous solution of sarcosine and an equimolar KOH was freeze-dried to give K sarcosinate. One mmole of the K salt dissolved in 20 ml MeOH was added to a MeOH solution of 1 mmole pyridoxal. The mixture, after sitrring 1 hr at room temperature, was evaporated to dryness under reduced pressure. Pale yellow glassy material was obtained and was submitted for NMR study after verification of purity by UV spectra.

**Cyclic Product from Histidine (Potassium 4-(3-Hydroxy-5-hydroxymethyl-2-methyl-4-pyridyl)-4,5,6,7-tetrahydropyrido[3,4-c]imidazole-6-carboxylate)**——One mmole of histidine hydrochloride was dissolved in MeOH containing two equimolar KOH. The K histidinate solution was added to one mmole MeOH solution of pyridoxal. The mixture immediately turned deep yellow but faded off in several hours. After stirring 24 hr, resulting colorless solution was concentrated in vacuum. White precipitate obtained was recrystallized twice form MeOH and twice from 80% MeOH to give white needles. *Anal.* Calcd. for $C_{14}H_{15}N_4K \cdot H_2O$: N, 15.54. Found: N, 15.60.

**Thiazolidine Derivative (2-(3-Hydroxy-5-hydroxymethyl-2-methyl-4-pyridyl)thiazolidine)**——In 10 ml MeOH, 1 mmole of cysteamine hydrochloride was dissolved and MeOH containing 1 equimolar KOH was added to the solution. After filtrating precipitated KCl, the filtrate was added to MeOH solution of 1 mmole pyridoxal. After stirring 1 hr at room temperature, the mixture was evaporated to dryness under reduced pressure. Obtained yellow powder was recrystallized three times from $CHCl_3$ to give colorless crystals. *Anal.* Calcd. for $C_{10}H_{14}O_2N_2S$: C, 53.07; H, 6.25; N, 12.38. Found: C, 53.08; H, 6.29; N, 12.62.

**Acknowledgement** The authors are indebted to Mr. T. Kondo of this Institute for NMR measurements and to the members of analytical laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, for elemental analysis. They also thank Dr. Ya. Karpeisky, Institute of Molecular Biology, Academy of Sciences of U.S.S.R. for discussion.

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*UDC 581.19 : 582.46 : 547.99*

# Sterische Struktur der Giftstoffe aus dem Fruchtfleisch von *Ginkgo biloba* L.

HIROSHI MORIMOTO, YUTAKA KAWAMATSU und HIROSADA SUGIHARA

*Forschungslaboratorien für Chemie, Takeda Chemische Industrien A.G.*[1)]

(Eingegangen am 7. Juni 1968)

Als Giftstoffe des Fruchtfleisches von *Ginkgo biloba* L., die starke Hautentzündung hervorrufen, wurden die Ginkgolsäure (I) und Bilobol (II) vor etwa vierzig Jahren von Kawamura isoliert,[2)] und deren Struktur mit Ausnahme der geometrischen Konfiguration an einer Doppelbindung in der Seitenkette von Furukawa aufgeklärt.[3–6)]

1) Standort: *Juso-Nishino-cho, Higashiyodogawa-ku, Osaka.*
2) J. Kawamura, *Japan J. Chem.*, 3, 89 (1928).
3) S. Furukawa, *Sci. Papers Inst. Phys. Chem. Res.*, **24**, 304 (1934).
4) S. Furukawa, *Sci. Papers Inst. Phys. Chem. Res.*, **24**, 314 (1934).
5) S. Furukawa, *Sci. Papers Inst. Phys. Chem. Res.*, **24**, 320 (1934).
6) S. Furukawa, *Sci. Papers Inst. Phys. Chem. Res.*, **26**, 178 (1935).