Synthesis of Optically Active α-Phenylpyridylmethanols with Cell Cultures of *Nicotiana tabacum*

Masumi TAKEMOTO,*,[a] Yuji MORIYASU,[b] and Kazuo ACHIWA*,[a]

School of Pharmaceutical Sciences (Laboratory of Medicinal Chemistry)[a] and Faculty of International Relations (General Education),[b] University of Shizuoka, 52-1 Yada, Shizuoka 422, Japan.
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We have synthesized optically active α-phenylpyridylmethanols by reduction or hydrolysis with cell cultures of *Nicotiana tabacum* or immobilized cells of *N. tabacum*.

Key words chiral α-phenylpyridylmethanol; *N. tabacum* immobilized cell; microbial reduction; microbial hydrolysis; benzoylpyridine

α-Pyridyl alcohols are intermediates of pharmacological interest,[1-3] and (+)-(*S*)-α-phenyl-2-pyridyl methanol (**2c**) itself has analgetic and anticonvulsant activities.[4] In a preceding paper,[5] we have reported the synthesis of optically active α-phenyl-4,3- or 2-pyridylmethanol (**2a—c**) by using fermenting baker's yeast (BY) [reduction of 4-, 3-, or 2-benzoylpyridine (**1a—c**) or asymmetric hydrolysis of racemic 4-, 3-, or 2-(α-acetoxybenzyl)pyridine (**3a—c**)]. In the case of the reduction of **1a**, BY could discriminate the phenyl group and pyridinyl group of **1a** in spite of the apparent stereochemical resemblance between the phenyl group and the pyridinyl group, affording (−)-α-phenyl-4-pyridylmethanol [(−)-**2a**] in high optical purity [free BY in water, 86% ee; immobilized BY (IMBY) in water, 84% ee; IMBY in hexane, 96% ee]. However, *meta* (**1b**) and *ortho* (**1c**) forms were less well discriminated by BY, which afforded the alcohols **2b** and **2c** in lower optical yields [free BY in water, (−)-**2b** (56% ee); (+)-(*S*)-**2c** (32% ee)]. Furthermore, in the case of asymmetric hydrolysis of **3a—c** by BY, the optical yields of the alcohols **2a—c** were much lower than those in the case of reduction with BY [free BY in water, (−)-**2a** (51% ee), (+)-**2b** (31% ee), (+)-(*S*)-**2c** (4% ee)].

In recent years, much attention has been paid to the ability of cultured plant cells to transform enantioselectively not only secondary metabolites, but also organic foreign substrates.[6-11] The biotransformation of the exogenous substrates by plant cell cultures can be summarized according to classes of chemical reactions as follows: 1) hydroxylation, 2) oxido–reduction between alcohols and ketones, 3) reduction of the carbon–carbon double bond, 4) glycosyl conjugation, 5) hydrolysis and 6) miscellaneous reactions. However, there have been few examples of biotransformations of organic foreign substrates. We have been interested in the feasibility of using plant cell cultures for the biotransformation of foreign substrates. In this study, we selected 4-, 3- or 2-benzoylpyridine (**1a—c**) and racemic 4-, 3-, or 2-(α-acetoxybenzyl)pyridine (**3a—c**) as organic foreign substrates and *N. tabacum* cells (NTC) as the plant cell culture, to see whether NTC can discriminate the phenyl group and pyridinyl group of **1a—c** (reduction of carbonyl group) and **3a—c** (hydrolysis), in spite of their apparent stereochemical resemblance, in the same way as BY. In this paper, we report the first synthesis of optically active α-phenylpyridylmethanols with NTC.

In this work, we used suspension-cultured cells which had originally been isolated from *N. tabacum* "Bright Yellow-2." Subculturing was performed every 7 d by transferring 1.3 ml of 1-week-old culture into 80 ml of fresh Murashige and Skoog's (MS) medium[12] containing 2 ppm of 2,4-dichlorophenoxyacetic acid (2,4-D) as an auxin and 3% sucrose. Incubation was done on a rotary shaker (95 rpm) at 25 °C in the dark.

The bioreduction of **1a—c** was performed by three methods, that is, [A] with freely suspended NTC in the stationary phase after 10 d of incubation (30—40 g of cells in MS medium 80 ml), [B] with immobilized NTC (*N. tabacum* cells only) in MS medium, and [C] with immobilized NTC (*N. tabacum* cells and culture broth) in MS medium. Immobilized cultured cells of *N. tabacum* (INTC) were prepared according to the following procedure. Method [B]: *N. tabacum* cells in the stationary phase after 10 d of incubation were separated by filtration. The separated cells (30—40 g) were mixed with 5% sodium alginate solution (300—400 ml). The resultant mixture was dropped into a 0.6% $CaCl_2$ solution (1000 ml) and rinsed with water to give INTC. INTC (including 6 g of NTC) was added to freshly prepared MS medium (80 ml per flask) containing 2 ppm of 2,4-D and 3% sucrose, and was shaken on a rotary shaker (95 rpm) in the dark for 25 °C. Method [C]: Freely suspended NTC [30—40 g of cells and MS medium] in the stationary phase after 10 d of incubation was mixed with 5% sodium alginate solution (300—400 ml). The subsequent procedure was the same as for method [B]. A substrate (30 mg) was added to the freely suspended NTC (6 g of NTC in 20 ml of MS medium) or INTC-MS medium (including 6 g of NTC, 80 ml per flask, method [B] or [C]) and the mixture was shaken on a rotary shaker (95 rpm) at 25 °C.

As shown in Fig. 1, the time courses of bioreduction of **1a** and **1b** with NTC (method [A]) and INTC (method [B]) were examined in terms of the chemical yields of **2a** and **2b**. The bioreduction of **1a** and **1b** with freely suspended NTC (method [A]) stopped before reaching 100% conversion, affording the alcohols **2a** and **2b** in low chemical yield and in 0% optical yield. We consider that the cells were killed by the substrate. In the case of INTC (method [B]), the biotransformation began after 10 d (**2a**) or 20 d (**2b**) and the rate of biotransformation accelerated

* To whom correspondence should be addressed.

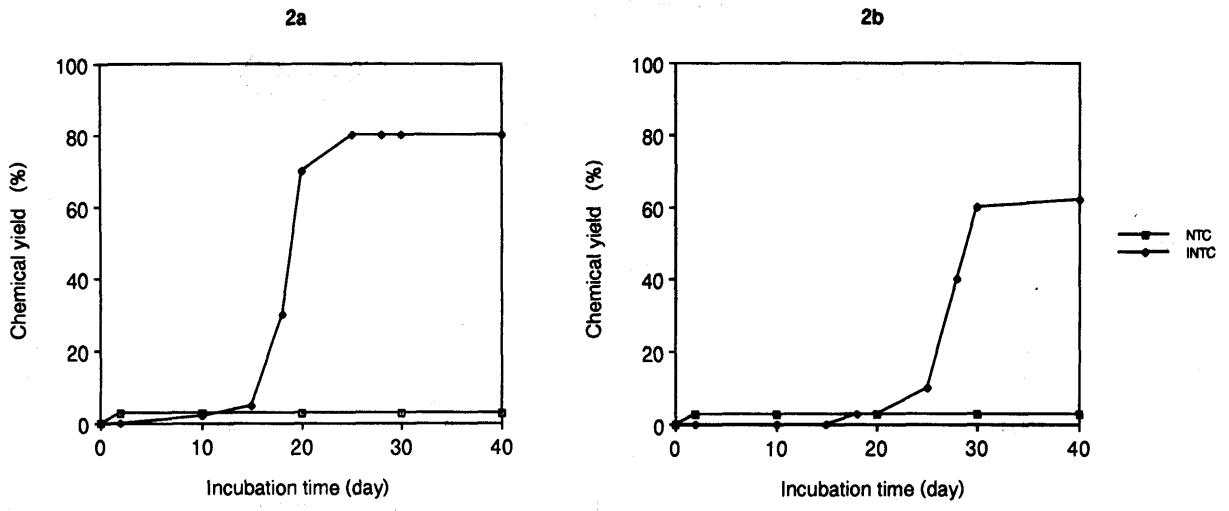


Fig. 1. The Time/Course of Chemical Yields (%) of **2a** and **2b** in the Bioreduction of **1a** and **1b** to **2a** and **2b** with NTC (Method [A]) or INTC (Method [B])

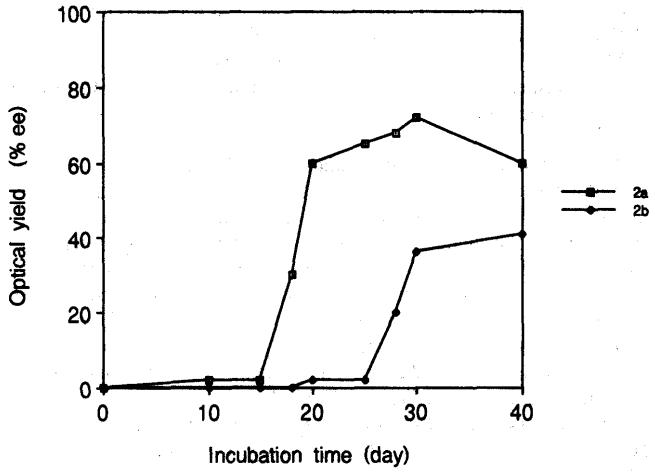


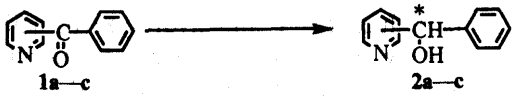
Fig. 2. The Time/Course of ee(%) in the Biotransformation of **1a** and **1b** to **2a** and **2b** with INTC (Method [B])

over 20—30 d. As shown in Fig. 2, the optical yields were very low at low conversion, but increased with increasing bioconversion, reaching a maximum of 72% ee (**2a**) and 41% ee (**2b**). As shown in Table 1, INTC (method [B]) enantioselectively bioreduced compounds **1a** and **1b** over 30—40 d at 25 °C to the corresponding (+)-α-phenyl-4-pyridylmethanol [(+)-**2a**[13]] and (+)-α-phenyl-3-pyridylmethanol [(+)-**2b**] with opposite stereochemistry compared to that with IMBY in optical yields of 72% and 41% and chemical yields of 80% and 62%, respectively. But, in the case of **1C**, the bioreduction was unsuccessful.

The results of another INTC (method [C]) are as shown in Table 1. The biotransformation proceeded at a faster rate than that with method [B] to afford **2a**—**c** in high chemical yields. Compound **1c** was enantioselectively bioreduced in 12 d at 25 °C to the corresponding (+)-α-phenyl-2-pyridylmethanol [(+)-(S)-**2c**[4]] in an optical yield of 48% and a chemical yield of 82%, much higher than those with IMBY. Method [C] seems to involve a greater influence of NAD^+-dependent alcohol dehydrogenase, not only in *N. tabacum* cells, but also in the culture broth.

Secondly, we tried the asymmetric hydrolysis of racemic 4-(α-acetoxybenzyl)pyridine (**3a**[14]), 3-(α-acetoxybenzyl)-

Table 1. Bioreduction of the Benzoylpyridines **1a**—**c** with NTC, INTC and IMBY



1a—**c** **2a**—**c**

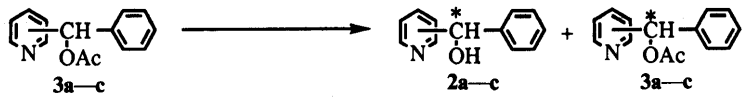
a: 4-, **b**: 3-, **c**: 2-

Substrate		Product	% Yield	% ee[a]	Time (d)
1a	NTC (A)	**2a**	3	0	2
1a	INTC (B)	(+)-**2a**	80	72	30
1a	INTC (C)	(+)-**2a**	79	71	15
1a[b]	IMBY	(−)-**2a**	86	84	2
1b	NTC (A)	**2b**	3	0	2
1b	INTC (B)	(+)-**2b**	62	41	40
1b	INTC (C)	(+)-**2b**	80	50	15
1b[b]	IMBY	(−)-**2b**	65	45	5
1c	NTC (A)	**2c**	3	0	2
1c	INTC (B)				40
1c	INTC (C)	(+)-**2c** (*S*)	82	48	12
1c[b]	IMBY	(+)-**2c** (*S*)	49	28	6

NTC (A): freely suspended *N. tabacum* culture (method [A]); INTC (B): sodium alginate-immobilized *N. tabacum* cells only (method [B]); INTC (C): sodium alginate-immobilized *N. tabacum* (cells and culture broth) (method [C]); IMBY: sodium alginate-immobilized baker's yeast. *a*) Optical yields were determined by HPLC analysis. **2a** (Chiralcel OB, 2-propanol/hexane = 2/3); **2b** (Chiralcel OB, 2-propanol/hexane = 2/3); **2c** (Chiralcel OJ, 2-propanol/hexane = 1/30). *b*) See ref. 5.

pyridine (**3b**) and 2-(α-acetoxybenzyl)pyridine (**3c**[15]) using INTC (method [B]). As shown in Table 2, the hydrolysis of racemic **3a**—**c** with INTC for 5 h at 25 °C gave the corresponding (−)-**2a** and (−)-**2b** with optical purities of 83% ee and 85% ee and chemical yields of 37% and 35%, and recovered acetate **3a** and **3b** with optical purities of 56% ee and 58% ee and chemical yields of 52% and 50%, respectively. In the case of the racemic acetate **3c**, the INTC hydrolysis was unsuccessful, affording the racemic alcohol **2c**. It is noteworthy that INTC enantioselectively hydrolyzed the racemic acetates **3a** and **3b** to the corresponding (−)-**2a** and (−)-**2b** in excellent optical yields of 83% ee and 85% ee as compared with IMBY (40% ee and 33% ee). Furthermore, it is interesting that hydrolysis of the racemic acetates **3a** and **3b** with INTC afforded (−)-**2a** and (−)-**2b**, whereas reduction of **1a** and

Table 2. Asymmetric Hydrolysis of the Acetates **3a—c** with INTC and IMBY



a: 4-, b: 3-, c: 2-

Substrate		Time (h)	Reacted alcohol (2a—c)		Recovered acetate (3a—c)	
			% Yield	% ee	% Yield	% ee[a]
3a	INTC	5	37	83 (−)	52	56
3a[b]	IMBY	48	30	40 (−)	51	23
3b	INTC	5	35	85 (−)	50	58
3b[b]	IMBY	48	18	33 (+)	54	10
3c	INTC	5	40	0	42	2
3c[b]	IMBY	48	30	0	40	2

INTC: immobilized *N. tabacum* cultures (cells) (method [B]). IMBY: immobilized baker's yeast. *a*) Optical yields were determined by HPLC analysis. **3a** (Chiralcel OJ, 2-propanol/hexane = 1/5). **3b** (Chiralcel OJ, 2-propanol/hexane = 2/3). **3c** (Chiralcel OJ, 2-propanol/hexane = 1/30). *b*) See ref. 5.

1b with INTC afforded (+)-**2a** and (+)-**2b** with the opposite stereochemistry.

We have established that *N. tabacum* culture has the ability to bioreduce **1a—c** and hydrolyze racemic **3a, b** enantioselectively, in spite of the apparent stereochemical resemblance between the phenyl group and the pyridyl group. Its capability for asymmetric hydrolysis is excellent.

Although bioreduction in the case of INTC required much longer reaction times than that with IMBY, the chemical and optical yields of the alcohols **2a** and **2b** with INTC were comparable to those obtained with IMBY. It is noteworthy that the bioreduction of **1a** and **1b** with INTC afforded the alcohols **2a** and **2b** with the opposite stereochemistry as compared with IMBY. Furthermore, immobilized NTC (method [C]) is a good system for the bioreduction of **1a—c** from the viewpoints of optical yield, chemical yield and reaction time.

Experimental

Melting points were determined on a micro-melting point apparatus (Yanagimoto) and are uncorrected. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. IR spectra were taken on JASCO IR-810 IR spectrophotometers and values are given in cm^{-1}. ^1H-NMR spectra were recorded on a JEOL JNM PMX 60SI (60 MHz) spectrophotometer in $CDCl_3$. Chemical shifts are given in δ (ppm) downfield from tetramethylsilane, and the abbreviations of signal patterns are as follows: s, singlet; d, doublet; t, triplet. High-performance liquid chromatography (HPLC) was carried out with a Waters 600E (ultraviolet detection) equipped with a column packed with Chiralcel OB (Daicel Chemical Industries Ltd., 2-propanol/hexane) or Chiralcel OJ (Daicel Chemical Industries Ltd., 2-propanol/hexane). Thin layer chromatography (TLC) was performed on silica gel (Kieselgel $60F_{254}$ on aluminum sheets, Merck). All compounds were located by spraying the TLC plate with a 10% solution of phosphomolybdic acid in ethanol and heating it on a hot plate. Preparative TLC was performed on preparative layer chromatography plates (Kieselgel $60F_{254}$ 2 mm and 0.5 mm, Merck). Column chromatography was performed on silica gel (Kieselgel 60, 70—230 mesh, Merck).

Cultivation of *N. tabacum* "Bright Yellow-2" *N. tabacum* "Bright Yellow-2" was subcultivated every 7 d by transferring 1.3 ml of 1-week-old culture into 80 ml of MS medium containing 0.2 ppm of 2,4-D and 3% sucrose (PH 5.8) on a rotary shaker (95 rpm) at 25 °C in the dark.

Preparation of Immobilized NTC Method [B]: NTC in the stationary phase (30—40 g, 10 d) were collected by filtration. The separated cells (30—40 g) were added to a 5% sodium alginate solution (300—400 ml), and the mixture was stirred until it became homogeneous. The sodium alginate mixture was added dropwise to 0.6% $CaCl_2$ solution (1000 ml) and rinsed with water to give INTC. INTC (including 6 g of NTC) was added to freshly prepared MS medium (80 ml per flask) containing 2 ppm of 2,4-D and 3% sucrose, and was shaken on a rotary shaker (95 rpm) in the dark at 25 °C for 2 d.

Method [C]: A 5% sodium alginate solution (300—400 ml) was added to freely suspended NTC in the stationary phase (80 ml of MS medium, 10 d). The subsequent procedure was the same as described for method [B].

Bioreduction of 1a—c with Method [A] A substrate **1a—c** (30 mg) was added to the suspension culture (20 ml) and the culture was incubated at 25 °C on a rotary shaker (95 rpm) in the dark. At the conclusion of the reaction, the incubation mixture was filtered and the filtered cells were washed with CH_2Cl_2. The filtrate was extracted with CH_2Cl_2, and the combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on SiO_2 with CH_2Cl_2 to give the corresponding phenylpyridylmethanol (**2a—c**). The reaction time, the chemical yield and the optical yield are listed in Table 1.

Biotransformation of Substrates (1a—c or 3a—c) with Method [B] or [C] A substrate (**1a—c** or **3a—c**) (30 mg) was added to precultured MS medium (80 ml) containing the immobilized cells, and the resultant cultures were incubated at 25 °C on a rotary shaker (95 rpm) in the dark. At the conclusion of the reaction, the mixture was separated by filtration and the immobilized cells were washed with CH_2Cl_2. The subsequent procedure was the same as described for Method [A]. The reaction time, the chemical yield and the optical yield (O.Y.) are listed in Tables 1 and 2.

(−)-**2a**: mp 155—156 °C. $[\alpha]_D^{20}$ −65.1 (c = 1.30, $CHCl_3$). O.Y. 83% ee. (lit.[13] mp 131—132 °C. $[\alpha]_D^{18}$ −55.5 (c = 3.66, $CHCl_3$).

(+)-**2a**: mp 155—156 °C. $[\alpha]_D^{20}$ +56.5 (c = 1.30, $CHCl_3$). O.Y. 72% ee. (lit.[13] mp 131—132 °C. $[\alpha]_D^{18}$ +52.4 (c = 2.08, $CHCl_3$).

(−)-**2b**: mp 80—81 °C. $[\alpha]_{20}^{D}$ −16.9 (c = 1.38, $CHCl_3$). O.Y. 85% ee. IR (KBr): 3346 (OH). *Anal.* Calcd for $C_{12}H_{11}NO$: C, 77.81; H, 5.99; N, 7.56. Found: C, 77.72; H, 5.85; N, 7.57. MS *m/z*: 185 (M^+). ^1H-NMR: 4.17 (1H, s, OH), 6.05 (1H, s, side chain H), 7.40 (1H, dd, $J_{4,5}$ = 7.6 Hz, $J_{5,6}$ = 3 Hz, C_5-H), 7.52 (5H, s, phenyl), 7.90 (1H, tt, $J_{4,5}$ = 7.6 Hz, $J_{2,4}$ = 2 Hz, $J_{4,6}$ = 2 Hz, C_4-H), 8.55 (1H, dd, $J_{4,6}$ = 2 Hz, $J_{5,6}$ = 3 Hz, C_6-H), 8.68 (1H, d, $J_{2,4}$ = 2 Hz, C_2-H).

(+)-**2b**: mp 80—81 °C. $[\alpha]_D^{20}$ +9.9 (c = 1.50, $CHCl_3$). O.Y. 50% ee. IR (KBr): 3346 (OH). *Anal.* Calcd for $C_{12}H_{11}NO$: C, 77.81; H, 5.99; N, 7.56. Found: C, 77.65; H, 5.87; N, 7.45. MS *m/z*: 185 (M^+). ^1H-NMR: 4.17 (1H, s, OH), 6.05 (1H, s, side chain H), 7.40 (1H, dd, $J_{4,5}$ = 7.6 Hz, $J_{5,6}$ = 3 Hz, C_5-H), 7.52 (5H, s, phenyl), 7.90 (1H, tt, $J_{4,5}$ = 7.6 Hz, $J_{2,4}$ = 2 Hz, $J_{4,6}$ = 2 Hz, C_4-H), 8.55 (1H, dd, $J_{4,6}$ = 2 Hz, $J_{5,6}$ = 3 Hz, C_6-H), 8.68 (1H, d, $J_{2,4}$ = 2 Hz, C_2-H).

(+)-**2c**: mp 64—65 °C. $[\alpha]_D^{20}$ +67.3 (c = 2.60, $CHCl_3$). O.Y. 48% ee. [lit.[4] mp 64—65 °C. $[\alpha]_D^{25}$ +17.2 (c = 1.54, $CHCl_3$), lit[16] (−)-**2c**: $[\alpha]_D^{25}$ −114.6 (c = 2.81, $CHCl_3$). O.Y. 93% ee.]

Time-Course Experiments on the Biotransformation of Substrates For the time-course experiments on the biotransformation with Method [A] or [B], a part of the incubation mixture was pippeted out and then extracted with EtOAc. The conversion ratios and optical yields were measured by HPLC analyses.

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References

1) Yale H. Y., "Pyridine and Its Derivatives," Part 2, ed. by Klingsberg E., Interscience Publishers, Inc., New York, 1961.
2) Spencer N., Papa D., Scheenk E., Sherlock M., *J. Am. Chem. Soc.*, **73**, 3856 (1951).
3) Ashton M. J., Ashford A., Loveless A. H., Riddel D., Salmon J., Stevenson G. V. W., *J. Med. Chem.*, **27**, 1245 (1984).
4) Frank E., Gearien J., Megahy M., Pokorny C., *J. Med. Chem.*, **14**, 551 (1971).
5) Takemoto M., Achiwa K., *Chem. Pharm. Bull.*, **42**, 802 (1994).
6) Kutney J. P., *Synlett.*, **1991**, 11.
7) Galun E., Aviv D., Dantes A., Freeman A., *Planta Med.*, **51**, 511 (1985).
8) Suga T., Hirata T., Izumi S., *Phytochemistry*, **25**, 2791 (1986).
9) Hamada H., Umeda N., Otsuka N., Kawabe S., *Plant Cell Rep.*, **7**, 493 (1988).
10) Naoshima Y., Akakabe Y., *J. Org. Chem.*, **54**, 4237 (1989).
11) Orihara Y., Noguchi T., Furuya T., *Phytochemistry*, **35**, 941 (1994).
12) Murashige T., Skoog F., *Physiol. Plant.*, **15**, 473 (1962).
13) Davis A. G., Kenyon J., Thanker K., *J. Chem. Soc.*, **1956**, 3394.
14) Muth C. W., Darlak R. S., Patton J. C., *J. Heterocycl. Chem.*, **9**, 1003 (1972).
15) Shono T., Ohmizu H., Kawakami S., *Tetrahedron Lett.*, **1979**, 4091; Crook K. E., McElvain S. M., *J. Am. Chem. Soc.*, **52**, 4006 (1930) [*Chem. Abstr.*, **24**, 5752 (1930)].
16) Seki M., Baba N., Oda J., Inouye Y., *J. Org. Chem.*, **48**, 1370 (1983).