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Asymmetric syntheses of nitroalkanols using *Pseudomonas* sp. lipase: a proposal for the selection of the solvent system of lipase-catalyzed transesterification

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Abstract

By lipase-catalyzed stereoselective transesterification using Amano AK from *Pseudomonas* sp., nitroalcohols such as 1-nitro-2-butanol and 1-nitro-3-methyl-2-butanol were synthesized enantioselectively with enantiomeric ratios (E values) of 20.9 and 12.5, respectively, in *n*-propyl ether. Various results were obtained during the lipase-catalyzed transesterification by changing the organic solvents that were used. The plots of the E values against the reciprocal of the dielectric constants (ε) of the various organic solvents produced a bell-shaped curve which had a maximum E value for *n*-propyl ether ($1/\varepsilon = 0.3$). The distance between the enzyme and the substrate might be changed in response to a change in the organic solvent.

Keywords: Lipase; Asymmetric synthesis; Nitro alcohol; Solvent effect; Transesterification

1. Introduction

Lipase is an enzyme which was discovered in the pancreatic juice in 1856 by Bernard [1], and it catalyzes the hydrolysis of the ester group in fats and oils. In spite of a long history, the biophysical study of lipases falls far behind that of other hydrolytic enzymes, for example, proteases. Brady et al. performed the first X-ray analysis of a lipase, that from *Rhizomuchor miehei*, in 1990 [2]. It was found that the lipase contained three amino-acid residues (Ser 144, His 257, and Asp 203) in the catalytic reaction center, similar to serine proteases. Lipases from the human pancreas [3,4] and *Geotrichum candidum* [4] also contained those residues. In this manner, the structure of the active catalytic center of lipases has been clarified, but the relationship between the active catalytic center and enzyme activity remains unknown.

The activity of an enzyme is high in aqueous solution. However, enzyme-catalyzed reactions may be carried out in organic solvents which are needed for organic syntheses [5] since (1) a reaction such as esterification, which does not occur in aqueous solution, is then possible, (2)

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the concentration of the substrate can be increased, and (3) purification of the product is easier. In a reaction mediated by organic solvents, a minimal amount of water is essential to hold the active conformation of the enzyme [6], because in an anhydrous system the enzyme is inactivated by collapse of its active conformation [5].

The stereoselectivity of an enzyme-catalyzed reaction is the reason for its efficiency [7–9]. Important factors, which influence the stereoselectivity and the catalytic activity of the solvent in lipase-catalyzed kinetic resolutions, are the dipole moment, dielectric constant, and hydrophobicity parameter (Log P) of the solvent. Fitzpatrick and Klibanov [10] reported that the large dipole moment of solvents lowers the protease-catalyzed stereoselectivity. They reported that an enantioisomer is incorporated preferentially into the active center of the enzyme in a solvent with a higher dipole moment, because a large dipole moment of the solvent changes the conformation of the enzyme. Nakamura et al. reported that two groups of solvents, cyclic and acyclic, had different effects on enantioselectivity [11,12].

We have reported the lipase-catalyzed stereoselective preparation of four nitroalcohols which are useful as synthetic building blocks for the preparation of anticancer agents and insect pheromones [13–15]. This paper reports the effect of organic solvents over the course of lipase-catalyzed transesterification of Amano AK from *Pseudomonas* sp., a significant relationship between the reciprocal of the dielectric constant ($1/\varepsilon$) of the organic solvent and the enantiomeric ratio (E value) of the products.

2. Results and discussion

1-Nitro-2-butanol (**1**), 1-nitro-2-pentanol (**2**), 1-nitro-3-methyl-2-butanol (**3**), and 1-nitro-2-hexanol (**4**) were synthesized using the Knoevenagel reaction and purified by vacuum distillation to a purity of 98%, 100%, 98%, and 98%, respectively. The transesterification of the four nitroalcohols with vinyl acetate in various organic solvents in the presence of a lipase from *Pseudomonas* sp. (Amano AK) produced the corresponding acetoxy adduct along with unreacted substrates. For example, a mixture of **1**, vinyl acetate, and the lipase Amano AK in n-propyl ether (water content = 1.0% v/v) was stirred for 5 h at 30°C. The resulting mixture was filtered and the filtrate was concentrated. The residue was subjected to column chromatography on silica gel to produce a 47% yield of (+)-1-nitro-2-butylacetate (**5**) and a 53% recovery of **1**. As shown in Fig. 1, the enantiomeric excess (ee) of the product ((+)-**5**) was determined to be 80% by ^{1}H NMR analysis using Eu(hfc)$_3$ as a chiral shift reagent. The recovered **1** which had a positive optical rotation was chemically converted by AcCl/pyridine into (−)-1-nitro-2-acetoxybutane, and its ee was determined to be 77% by ^{1}H NMR analysis, δ; 3.9 and 4.0 ppm (singlet at *R*-Ac and *S*-Ac). The enantiomeric ratio (E value) was calculated from the ee values of the product

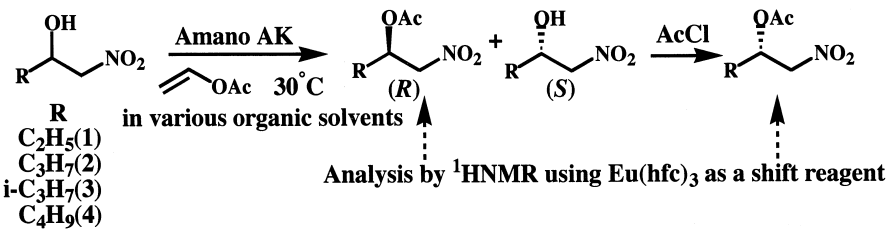

Fig. 1. Reaction conditions and analysis of enantiomer excess.

Table 1
Ee and *E* values of nitroalcohols

R		Solvents					
		Dioxane	THF	Benzene	AcOEt	Hexane	*n*-Propyl ether
C_2H_5 (**1**)	OH (ee)	40.6	41.4	69.8	37.8	78.0	77.4
	OAc (ee)	53.4	47.2	47.6	87.6	61.4	80.0
	E value	4.8	4.1	5.6	21.9	9.6	20.9
C_3H_7 (**2**)	OH (ee)	3.6	0.2	5.4	1.0	4.6	11.0
	OAc (ee)	9.2	4.2	13.8	17.4	0.4	2.6
	E value	1.2	1.1	1.4	1.4	1.0	2.1
i-C_3H_7 (**3**)	OH (ee)	49.8	61.2	65.0	34.6	78.0	15.4
	OAc (ee)	1.0	9.2	15.4	12.0	49.8	83.0
	E value	1.3	1.9	2.4	1.7	6.7	12.5
C_4H_9 (**4**)	OH (ee)	4.0	19.2	21.6	13.0	7.2	47.6
	OAc (ee)	5.0	16.0	14.2	9.8	39.4	12.8
	E value	1.2	1.6	1.6	1.4	2.5	3.4

and the remaining starting material [16]. Consumption of substrates was confirmed by gas chromatography.

As shown in Table 1, the *E* values varied with the solvent. When **1**, **2**, **3**, and **4** were employed as the substrate in *n*-propyl ether, the

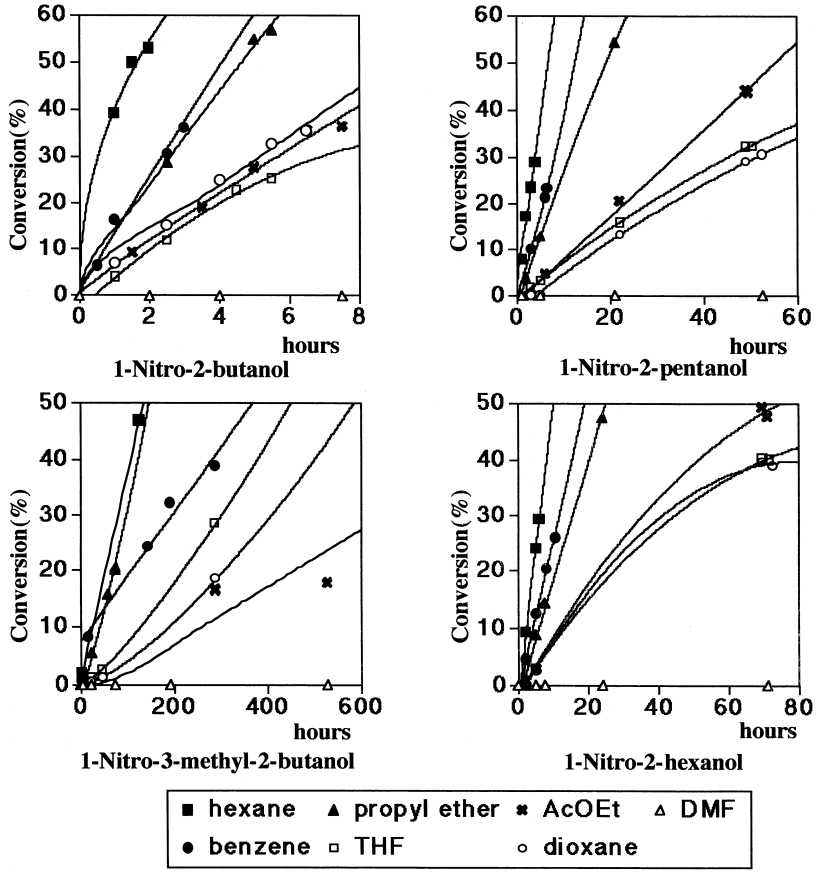

Fig. 2. Time course of transesterification.

best E values were 21.9, 2.1, 12.5, and 3.4, respectively.

Plots of the conversion against the reaction time of four nitroalcohols produced by the lipase-catalyzed transesterification are shown in Fig. 2. The conversion was measured by gas chromatography.

The longer the carbon chain of the substrate, the slower the reaction rate. The reaction rate of **3**, which has the most crowded molecular structure among these four compounds, was the slowest. The extent of change of the reaction rate for each substrate in the different solvents was approximately equal. The reaction rate of the lipase-catalyzed transesterification was faster in less polar solvents such as hexane or benzene, and slower in highly polar solvents such as THF or DMF.

We studied the correlation of the E value with other parameters, Log P (the hydrophobicity parameter which is the logarithm of the partition coefficient of a solvent between octanol and water [17]), ε (dielectric constant), and $1/\varepsilon$.

Nakamura et al. also studied a relationship between the E value and Log P for the lipase-catalyzed transesterification of 1-nitro-2-propanol in various organic solvents: the stereoselectivity of the reaction in a series of cyclic solvents was more sensitive to the E value than that in the acyclic solvents. The curve for the cyclic solvent group reached a maximum at THF whereas the curve for the acyclic group did not have a clear maximum. However, this relationship was not reproduced in our results as shown in Fig. 3.

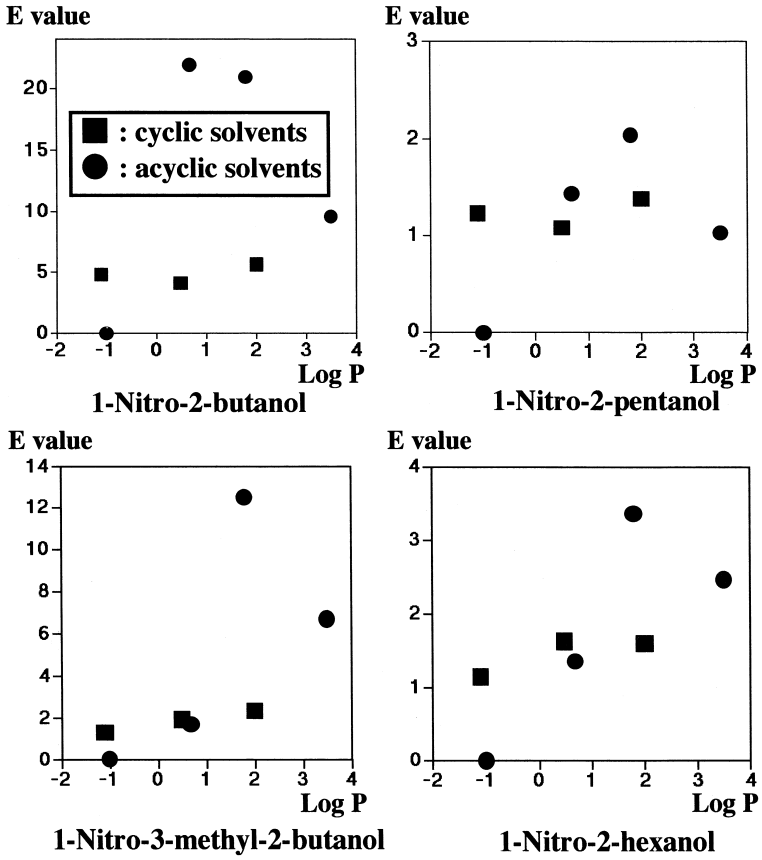

Fig. 3. Relationship between Log P of various organic solvents and the E value of the synthesized nitro alcohols.

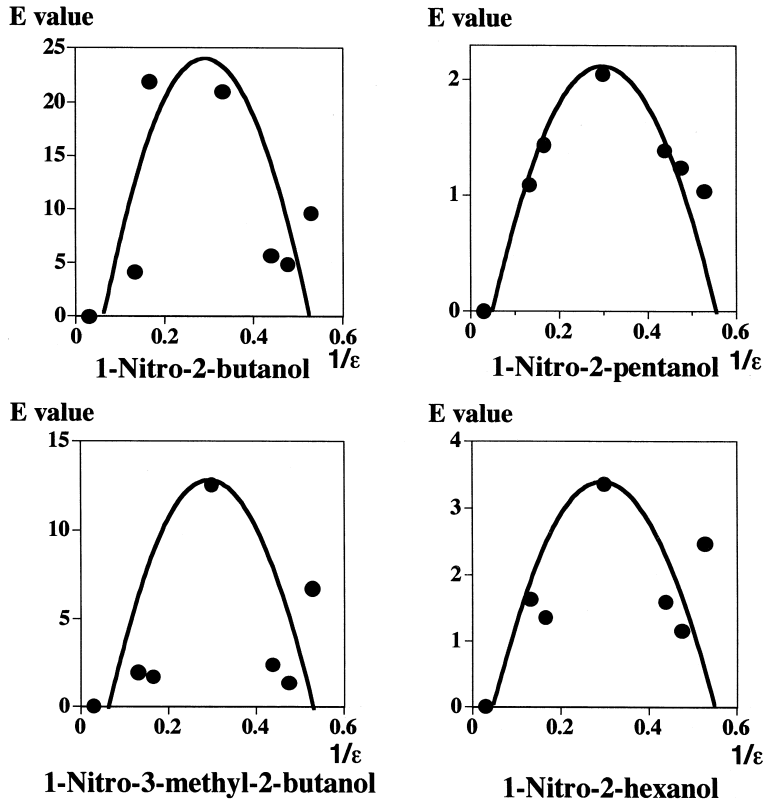

Fig. 4. Relationship between $1/\varepsilon$ of various organic solvents and the E value of the synthesized nitro alcohols.

No significant correlation of the stereoselectivity (E value) with the dielectric constant (ε) of the organic solvents was observed. The plots of the E value against the reciprocal of the ε of the organic solvents showed a bell-shaped curve with a maximum value in *n*-propyl ether ($1/\varepsilon = 0.3$) (Fig. 4), with the exception of transesterification of **1**, for which the E value was maximum value in ethyl acetate ($1/\varepsilon = 0.17$).

Although the significance of the physical properties of $1/\varepsilon$ is unknown, $1/\varepsilon$ can be considered as a unit of electrical resistance. The plots of the E value against $1/\varepsilon$ showed a smooth curve using the nitroalcohols as substrates, which suggests the importance of electrostatic interaction between the substrate and the enzyme. The E value decreased when either $1/\varepsilon$ was more than around 0.4 or less than around 0.2. The charge and the polarity of the enzyme and the substrate might change dynamically with a change in the $1/\varepsilon$ value of the organic solvent. This work presents a guide for the selection of the solvent to be used in lipase-catalyzed high-selective transesterification reactions. In many cases, *n*-propyl ether is recommended.

3. Conclusion

In the stereoselective syntheses of nitroalcohols of various carbon chain lengths using lipase-catalyzed transesterification, a correlation between the enantioselectivity of nitroalcohol, and the reciprocal of the dielectric constant of the solvent is observed.

4. Experimental

4.1. Instruments

^{1}H NMR spectra were recorded on a JEOL EX-270 spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as the internal reference. IR spectra were recorded on a Hitachi 270-30 infrared spectrometer. Optical rotation was measured with a Horiba SEPA-200 polarimeter. Gas-chromatographic analyses were performed using a Shimadzu gas chromatograph Model GC-9A equipped with an OV101 column.

4.2. Materials

Organic reagents and lipases were purchased from commercial sources unless otherwise indicated.

4.3. 1-Nitro-2-butanol (*1*)

Nitromethane (31.7 g, 0.52 mol) was added dropwise to a mixture of propionaldehyde (15.0 g, 0.26 mol), potassium fluoride (0.66 g, 1.1 mmol), and 460 ml of isopropyl alcohol at 30°C over 30 min. The mixture was stirred for 3 h at 35°C, and then KF was removed by filtration. The filtrate was poured into 100 ml of water and the organic material was extracted with ether (80 ml × 3). The combined organic layer was washed with 0.9% aqueous NaCl (80 ml × 3), dried over anhydrous sodium sulfate, and evaporated in vacuo. The residue was distilled at 67.0°C/1.3 mmHg to afford 1-nitro-2-butanol (21.1 g) in a 68% yield.

^{1}H NMR ($CDCl_3$): δ; 1.03 (t, 3H, $J = 7.26$ Hz), 1.59 (dq, 2H, $J = 7.26$ and 7.26 Hz), 2.59-2.62 (br, 1H), 4.27–4.34 (br, 1H), and 4.39–4.48 (m, 2H). IR (neat): 3450, 1560, and 1390 cm^{-1}. Anal. Calcd for $C_4H_9NO_3$: C, 40.33; H, 7.62; N, 11.76%. Found: C, 40.21; H, 7.59; N, 11.93%.

4.4. 1-Nitro-2-pentanol (*2*)

Bp: 91.0°C/1.8 mmHg, Yield: 87%

^{1}H NMR ($CDCl_3$): δ; 0.97 (t, 3H, $J = 7.26$ Hz), 1.42–1.46 (m, 4H), 2.58 (br, 1H), 4.34 (s, 1H), and 4.40–4.46 (m, 2H). IR (neat): 3450, 1555, and 1389 cm^{-1}. Anal. Calcd for $C_5H_{11}NO_3$: C, 45.10; H, 8.33; N, 10.52%. Found: C, 45.28; H, 8.30; N, 10.59%.

4.5. 1-Nitro-3-methyl-2-pentanol (*3*)

Bp: 79.5°C/2.8 mmHg, Yield: 77%

^{1}H NMR ($CDCl_3$): δ; 0.99 (d, 3H, $J = 6.92$ Hz), 1.00 (d, 3H, $J = 6.60$ Hz), 1.81 (ddq, 1H, $J = 6.91$, 6.60, and 5.94 Hz), 2.51 (d, 1H, $J =$ 4.61 Hz), 4.10–4.13 (m, 1H), and 4.37–4.51 (m, 1H). IR (neat): 3500, 1560, and 1390 cm^{-1}. Anal. Calcd for $C_5H_{11}NO_3$: C, 45.10; H, 8.33; N, 10.52%. Found: C, 45.03; H, 8.34; N, 10.45%.

4.6. 1-Nitro-2-hexanol (*4*)

Bp: 85.5°C/1.1 mmHg, Yield: 72%

^{1}H NMR ($CDCl_3$): δ; 0.93 (t, 3H, $J = 7.26$ Hz), 1.23–1.58 (m, 6H), 2.49 (d, 1H, $J = 4.62$ Hz), and 4.30–4.48 (m, 2H). IR (neat): 3500, 1562, and 1390 cm^{-1}. Anal. Calcd for $C_6H_{13}NO_3$: C, 48.97; H, 8.90; N, 9.52%. Found: C, 48.85; H, 8.92; N, 9.45%.

4.7. Lipase-catalyzed transesterification of 1-nitro-2-butanol

A mixture of 1-nitro-2-butanol (**1**, 2.86 mmol), vinyl acetate (17.5 mmol), and the lipase (dry Amano AK, 200 mg) in *n*-propyl ether (10 ml: water content = 1.0% v/v) was stirred for 5 h at 30°C. The reaction was followed by gas chromatography using a column of OV101 (Injection Temp.; 185°C, Column Temp.; 85°C, Carrier gas; N_2, Detector; FID). The resulting mixture was filtered and the filtrate was concentrated. The residue was subjected to column chromatography on a silica gel with a 4 to 1 ratio of hexane and ethyl acetate as an eluent to afford **5** in a 37% yield in an 88% ee as determined by ^{1}H NMR analysis using Eu(hfc)$_3$ as a chiral shift reagent. The remaining **1** was recovered.

Under a N_2 atmosphere, acetyl chloride (1.4 ml, 19.7 mmol) was added dropwise to a mixture of dry pyridine (1.4 ml, 17.5 mmol), which was stirred for 2 h, to recover **1** (50.0 mg, 0.42 mmol), and dry diethyl ether (10 ml) at room temperature. The solution was poured into 50 ml of water and the organic materials were extracted with ether (80 ml × 3). The combined organic layer was washed with 1 N hydrochloric acid (50 ml × 3), 0.9% NaCl (80 ml × 3), dried over anhydrous sodium sulfate, and evaporated in vacuo. The residue was subjected to column chromatography on a silica gel with a 4 to 1 ratio of hexane and ethyl acetate as an eluent to afford 1-nitro-2-butyl acetate in a 89% yield in a 38% ee as determined by ^{1}H NMR analysis using Eu(hfc)$_3$ as the chiral shift reagent.

*4.8. 1-Nitro-2-butyl acetate (**5**)*

^{1}H NMR ($CDCl_3$): δ; 0.98 (dd, 3H, $J = 7.58$ and 7.26 Hz), 1.72 (dddd, 2H, $J = 7.58$, 7.26, 6.60, and 6.27 Hz), 2.08 (s, 3H), 4.57 (dd, 2H, $J = 12.87$ and 1.32 Hz), and 5.41 (dddd, 1H, $J = 12.87$, 6.60, 6.27, and 5.61 Hz). IR (neat): 1760, 1568, and 1388 cm^{-1}. Anal. Calcd for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69%. Found: C, 44.58; H, 6.94; N, 8.80%.

*4.9. 1-Nitro-2-pentyl acetate (**6**)*

^{1}H NMR ($CDCl_3$): δ; 0.93 (t, 3H, $J = 7.34$ Hz), 1.32–1.43 (m, 1H), 1.64 (d, 2H, $J = 5.93$ Hz), 2.04 (s, 3H), 4.49 (d, 2H, $J = 5.93$ Hz), and 5.43 (dt, 1H, $J = 5.93$ and 5.61 Hz). IR (neat): 1758, 1565, and 1383 cm^{-1}. Anal. Calcd for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 8.00%. Found: C, 48.15; H, 7.51; N, 7.89%.

*4.10. 3-Methyl-1-nitro-2-butyl acetate (**7**)*

^{1}H NMR ($CDCl_3$): δ; 0.98 (dd, 6H, $J = 6.92$ and 6.60 Hz), 2.01 (m, 1H), 2.08 (s, 3H), 4.53 (dd, 2H, $J = 6.93$ and 2.96 Hz), and 5.36 (ddd, 1H, $J = 6.93$, 4.62, and 0.99 Hz). IR (neat): 1758, 1565, and 1383 cm^{-1}. Anal. Calcd for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 8.00%. Found: C, 48.16; H, 7.51; N, 8.15%.

*4.11. 1-Nitro-2-acetoxyhextane (**8**)*

^{1}H NMR ($CDCl_3$): δ; 0.91 (dd, 3H, $J = 6.93$ and 6.60 Hz), 1.35 (br, 4H), 1.64 (ddd, 2H, $J = 7.59$, 6.93, and 6.60 Hz), 2.07 (s, 3H), 4.52 (d, 2H, $J = 5.61$ Hz), and 5.45 (ddd, 1H, $J =$ 6.93, 6.60, and 6.27 Hz). IR (neat): 1760, 1568, and 1388 cm^{-1}. Anal. Calcd for $C_8H_{15}NO_4$: C, 50.78; H, 7.99; N, 7.40%. Found: C, 51.03; H, 8.01; N, 7.30%.

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